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Progress Report

DEVELOPMENT OF SAMPLING METHODS FOR NATURAL POPULATIONS OF THE DOUGLAS-FIR TUSSOCK MOTH

by

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SUMMARY

Studies were conducted in the summer of 1966 to develop reliable methods for estimating populations of the Douglas-fir tussock moth. Sampling methods are needed to evaluate future field tests of a virus spray and to provide basic quantitative data for ecological studies. Populations in an outbreak were sampled bi-weekly over most of one generation. A complete collapse of the outbreak by the end of the larval stage prevented sampling of the pupal or adult stages. Major results of the studies are listed below:

1. Egg masses were most abundant in the lower half and on the inside of the tree crown. Number of eggs on a one-acre study block can be estimated with a standard error of 20 percent of the mean from 200 whole-branch samples.

2. Larvae in all stages were successfully counted on short branch samples collected in a special muslin net. Larvae were distributed throughout the foliated portion of the tree, but density was higher in the top of the tree than in other parts of the crown. Branch samples from the mid-crown were representative of the larval population in the whole tree. Outbreak populations on a one-acre study block can be estimated from about 60 branch samples taken from 20 trees. For the same accuracy, endemic populations may require several times as many samples.

3. Tentative sequential sampling plans have been prepared which will minimize work where classification of populations into light, medium, or heavy infestation classes is acceptable. Additional study is needed, however, to perfect this method for sampling the tussock moth.

4. Total branch area on white fir is significantly correlated with tree diameter and can be used to estimate absolute numbers of larvae. In average stands of young white fir outbreak populations of the tussock moth may total over a million insects per acre.

5. The tussock moth population in the outbreak studied declined over a 10-week period from about 50 larvae to less than 1 larva per 1,000 sq.in. of branch area. A life table analysis revealed that virus disease and parasites accounted for about 64 percent of the decline; predation, starvation, and loss from dispersal accounted for 34 percent. Survival of small larvae had the most influence on ultimate larval survival.

6. Further investigations are needed to perfect sampling procedures, especially at low level populations, so that long term ecological studies of population trends in different areas can be developed.

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INTRODUCTION

A major effort is underway to perfect biological methods for controlling outbreaks of the Douglas-fir tussock moth, Hemerocampa pseudotsugata McD. Currently, emphasis is on the development for practical use of a nucleopolyhedrosis virus which commonly infects natural populations of the tussock moth and is a leading factor involved in the collapse of outbreaks. A prospectus was prepared in 1966 by the Pacific Northwest Forest and Range Experiment Station and Region 6[1/] which carefully outlined the research required before a virus control could be made operational.

Item 4C of the Prospectus pointed out the necessity of having reliable procedures for sampling field populations of the tussock moth so that future virus tests can be properly evaluated. Field experiments will test dosage rates and application techniques to determine the most effective methods for treating outbreaks. Determination of population size and changes that occur after virus treatment on various test blocks will be a vital part of the evaluation and will require precise methods of measurement.

Accurate estimation of insect numbers is also fundamental for the analysis of population processes which must be understood before injurious forest insects can be regulated. Development of quantitative techniques is, therefore, for many reasons a logical first step to take in the study of a pest population. This report explains the procedures used and the results from one season's analysis of an outbreak tussock moth population. Because the results are based on the study of only one generation at a single location they are not conclusive; however, they show that tussock moth populations can be accurately measured in the field and that further studies of their dynamics will certainly be profitable.

1/ Prospectus for a Joint-Administration Effort to Develop and Test a Method for Using a Nuclear-Polyhedrosis Virus Against the Douglas-fir Tussock Moth. Unpublished. Portland, Oregon. March 23, 1966.

Two noteworthy items of major importance should be mentioned at the outset: First, a late spring frost at the study site killed back most of the new growth on white fir and most trees did not initiate a second flush. This obviously had some behavioral and nutritional effects on tussock moth larvae, especially the early instars which normally feed extensively on new growth. Second, the outbreak collapsed almost completely by the sixth instar so that virtually no pupation was observed in the field. It was, therefore, impossible to get an estimate of this stage or to develop procedures for pupal sampling.

Many of the approaches and procedures used in the course of this study have been outlined by Morris (1955). His paper laid the groundwork for defoliator sampling and it was drawn upon heavily for ideas and techniques of analysis.

OBJECTIVES

The primary purpose for the field sampling studies in 1966 was:

1. To develop reliable sampling techniques for various life stages and different population levels of the tussock moth.

2. To determine concurrently survival values, mortality factors and trend of the outbreak.

METHODS

Location and Description of Study Area

The study area was a 450-acre infestation of the Douglas-fir tussock moth in white fir, Abies concolor (Gord. and Glend.) Lindl. Most of the infestation is in Sections 27 and 34, R.12 E., T.48 N., within the general boundaries of the Modoc National Forest in Modoc County, California (Figure 1). The forest type is predominantly an overstory of old-growth ponderosa pine, Pinus ponderosa Laws., in combination with a heavy understory of white fir. The bulk of white fir is in the 20- to 35-foot height class although some trees may reach 80 feet in height (Figure 2). Most of the actual infested timberland is owned by Weyerhaeuser Company. Forest Service ownership is restricted largely to fringes of the infestation and treeless meadows.

Study Blocks

Eight study blocks of about one acre in size were located in the infestation. Four blocks (1, 2, 3, and 8) were established in the center zone of heavy defoliation and four blocks (4, 5, 6, and 7) in transition zones of lesser defoliation (Figure 1). Blocks situated in varying infestation intensities covered a wide range of population levels which was useful for evaluating precision of estimate and required sample size for different conditions.

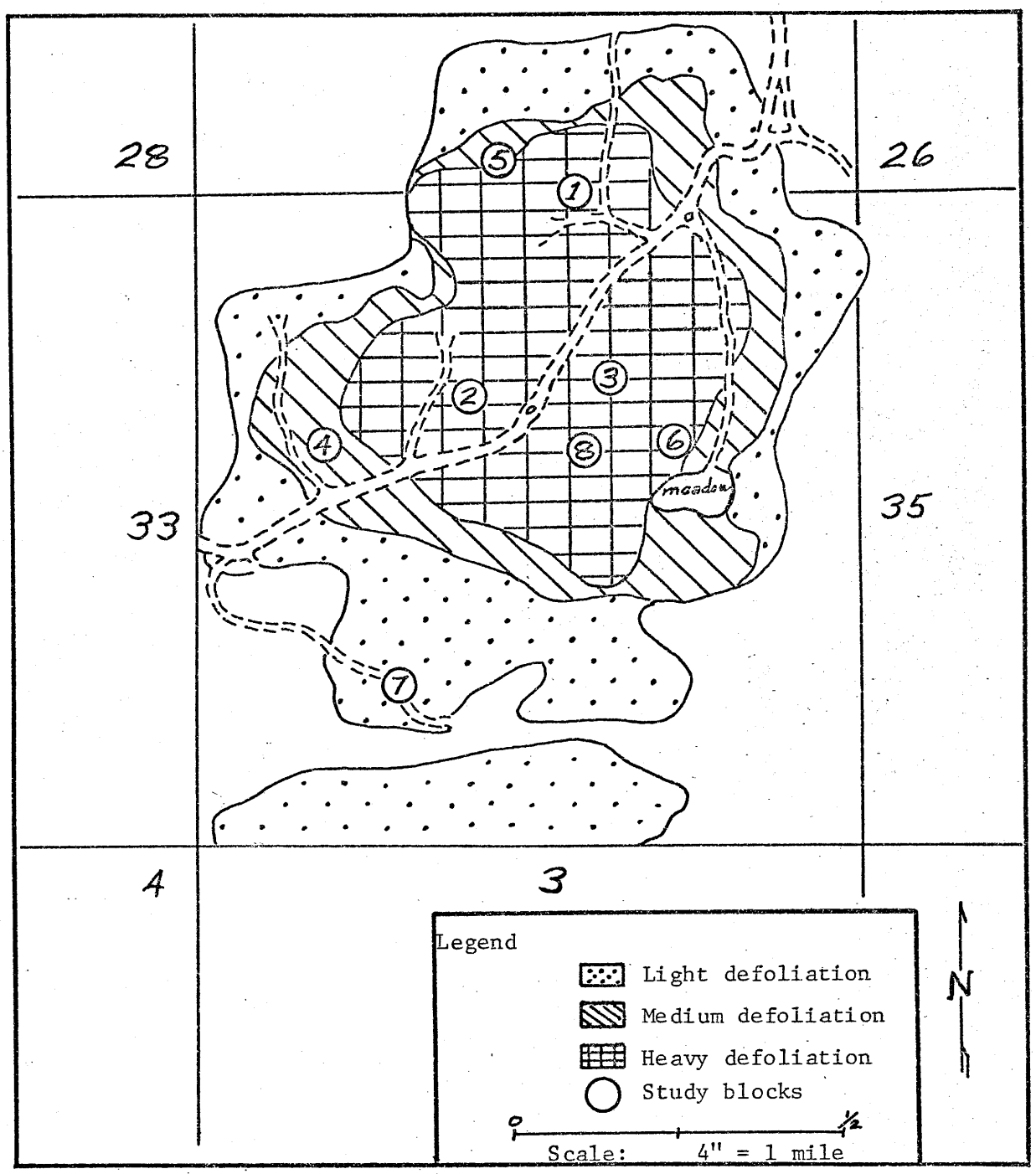

Figure 1.--Map of tussock moth defoliation and location of study blocks in T. 48 N., R. 18 E., Modoc County, California.

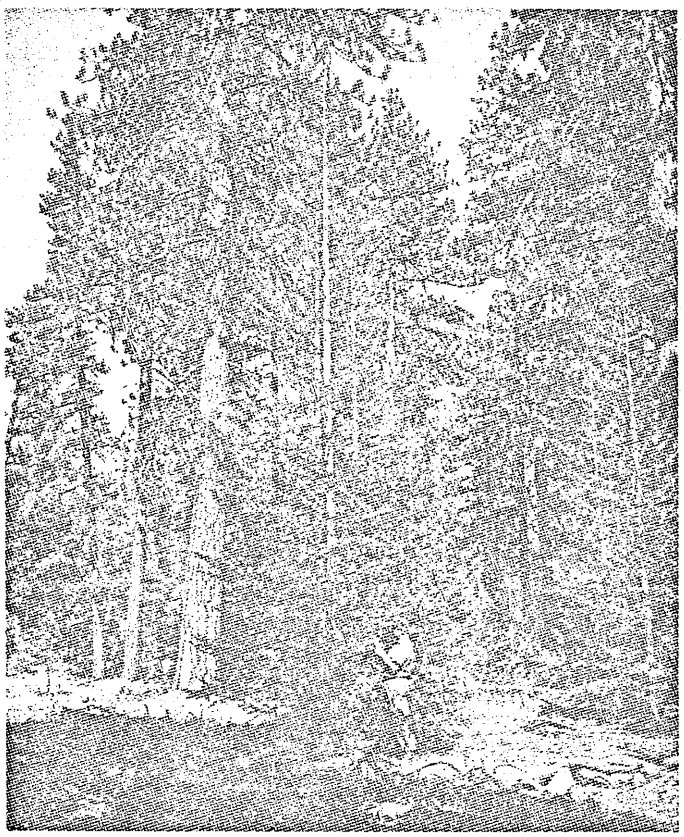

Figure 2. Defoliation by the Douglas-fir tussock moth on white fir at the Corral Creek study site.

Study Trees

Ten white fir trees were selected in each block and successive examinations were made during the period of insect activity. Trees were between 20 and 35 feet in height and accessible to sampling from a truck-mounted aerial ladder. In areas of heavy defoliation individuals with adequate foliage available for sampling were favored. Study trees were not adjacent to each other nor in a cluster, but were distributed over the site in a manner that seemed to be representative of the block.

Sampling Unit

The basic sample unit was a whole branch for eggs and a foliated section at least 15 inches in length for larvae. Number of egg masses, eggs or larvae were counted on each sample unit and related to the area of the branch sample occupied by foliage. Foliage area was determined by measuring the total length and the widest point of the sample and by calculating the area of the two included triangles. For analysis, all insect numbers were reduced to a common unit of area, 1,000 sq.in. This was done using the following formula:

$$\text{Insects/1,000 sq.in.} = \frac{\text{Number counted on sample} \times 2{,}000}{\text{Sample Lt} \times \text{Maximum width of sample}} \quad \ldots\ldots(1)$$

Mechanics of Sampling

Eggs

Since oviposition frequently occurs on bare branches near the bole, whole branches had to be sampled in the egg stage. Masses were counted on each branch and then removed and stored in plastic cups until processed in the laboratory. Eggs were separated from the mass by agitating in a 2 percent Clorox solution so that individual eggs could be counted.

Larvae

Short sections of foliage were used for sampling larval stages because of the difficulty in securing all larvae from a complete branch. It was assumed that most larvae were on foliated branches and, thus, had an equal chance of being sampled. Sample branches were collected in a white muslin cloth net, approximately 26x30 inches in dimension. The two opposite short sides were attached to dowels about 33 inches long, leaving seven inches for a handle on each side. Collection was made by quickly inserting the handles with muslin under the branch and enveloping the sample in the net. Nervous larvae that spun down or dropped from the branch as it was disturbed were caught in the net.

The branch sample was then clipped from the tree and momentarily wrapped in the net until it could be examined. Larvae were tallied by spreading the net on the ground, holding the branch sample over it and sharply rapping the branch with a stick so that all larvae were dislodged from the foliage onto the white muslin where they were counted. The procedure of sampling larvae is illustrated in Figures 3, 4, 5, and 6.

Within-tree Sampling

As a means of investigating vertical and lateral distribution of the tussock moth in the crown, foliage samples were taken from six different locations in each study tree.

Crown levels

Total bole length was ocularly divided into thirds so that the top one-third represented the upper crown, the middle one-third the mid-crown and the lower one-third the lower crown. Two branches were then sampled in each of the three crown levels.

Lateral position

Lateral distribution of larvae was studied by taking one of the foliage samples at each crown level from the end of the branch (outside crown) and the other from a side branch at least 17 inches inward from the end of the main branch (inside crown). This scheme insured all foliage an opportunity to be sampled.

Analysis of Variance

One-acre study blocks located through the infestation permitted several population densities to be sampled, each as a separate unit that might correspond to a treatment block in a field virus test. As already mentioned, samples were stratified by different levels and horizontal positions in the tree and by different trees in the block so that these could be analyzed for their contribution to variance. One egg sample and four separate larval samples were taken from each block over the course of the summer. Data from each sample period were ultimately examined by an analysis of variance employing the skeleton analysis shown in Table 1.

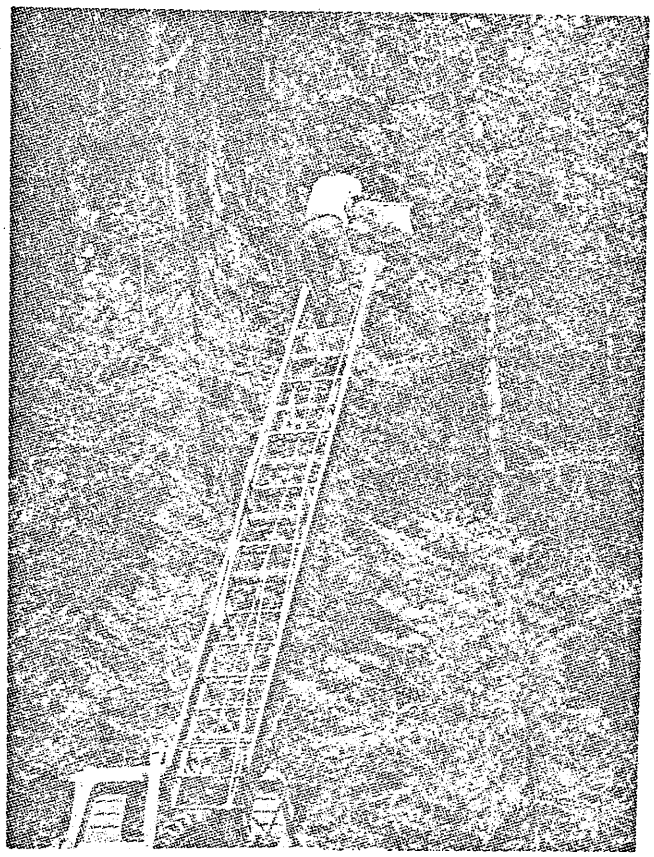

Figure 3. Sampling the crown of white fir from a truck-mounted aerial ladder.

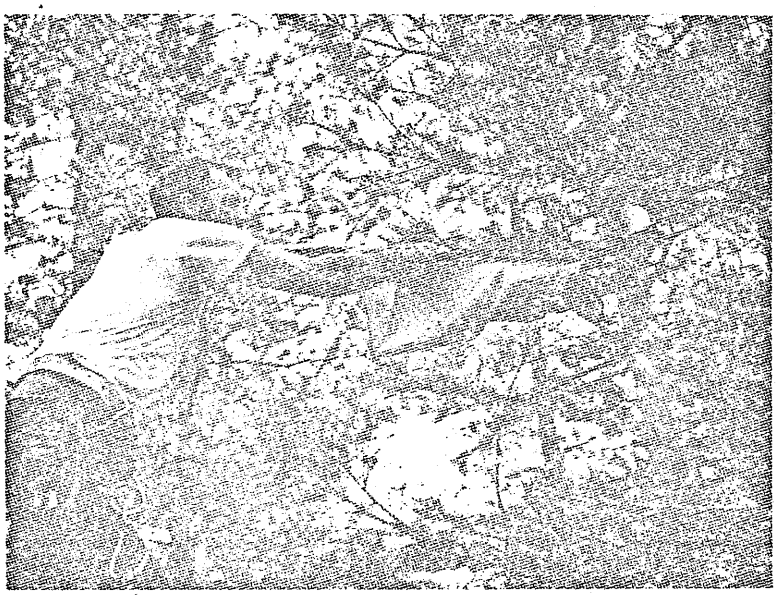

Figure 4. Enclosing sample in a muslin net to capture all larvae before clipping the branch.

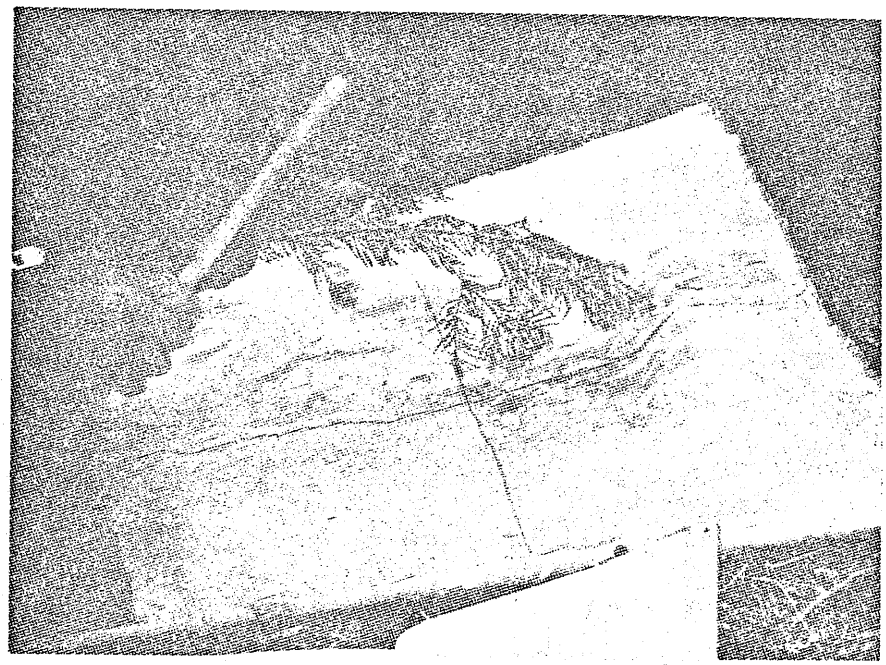

Figure 5. Rapping branch sample over muslin net to dislodge larvae.

Figure 6. Counting larvae on the net that were dislodged from the branch sample.

Table 1.--Skeleton analysis of variance used for study of variation in data collected during each sampling period

Source of Variation	DF	
Among blocks	7	
Within blocks	472	
Trees		72
Crown level		2
Lateral position		1
Residual		397
Total	479	

Tree Foliage Distribution

Fifteen trees similar in diameter and height to the sample trees were felled for examining crown characteristics. Every fourth branch was sampled and measured to determine branch area occupied by foliage and its distribution in the tree.

RESULTS

Sources of Variation

Analysis of variance assumes that variance has a normal distribution about the mean. If this condition does not exist then variance should be stabilized by a transformation before the data are analyzed by normal statistics. When the mean and variance from each block sample are plotted on a log/log scale it becomes obvious that the variance is not distributed by chance, but is strongly dependent on the mean (Figure 7). The plotted points, especially those with a mean over ten, fall roughly along or above the line having a slope of 2 where the variance approximates the square of the mean. For this situation, a logarithmic transformation is usually appropriate. All data were accordingly transformed by $y = \log (X + 1)$ to stabilize variance before performing the analysis.

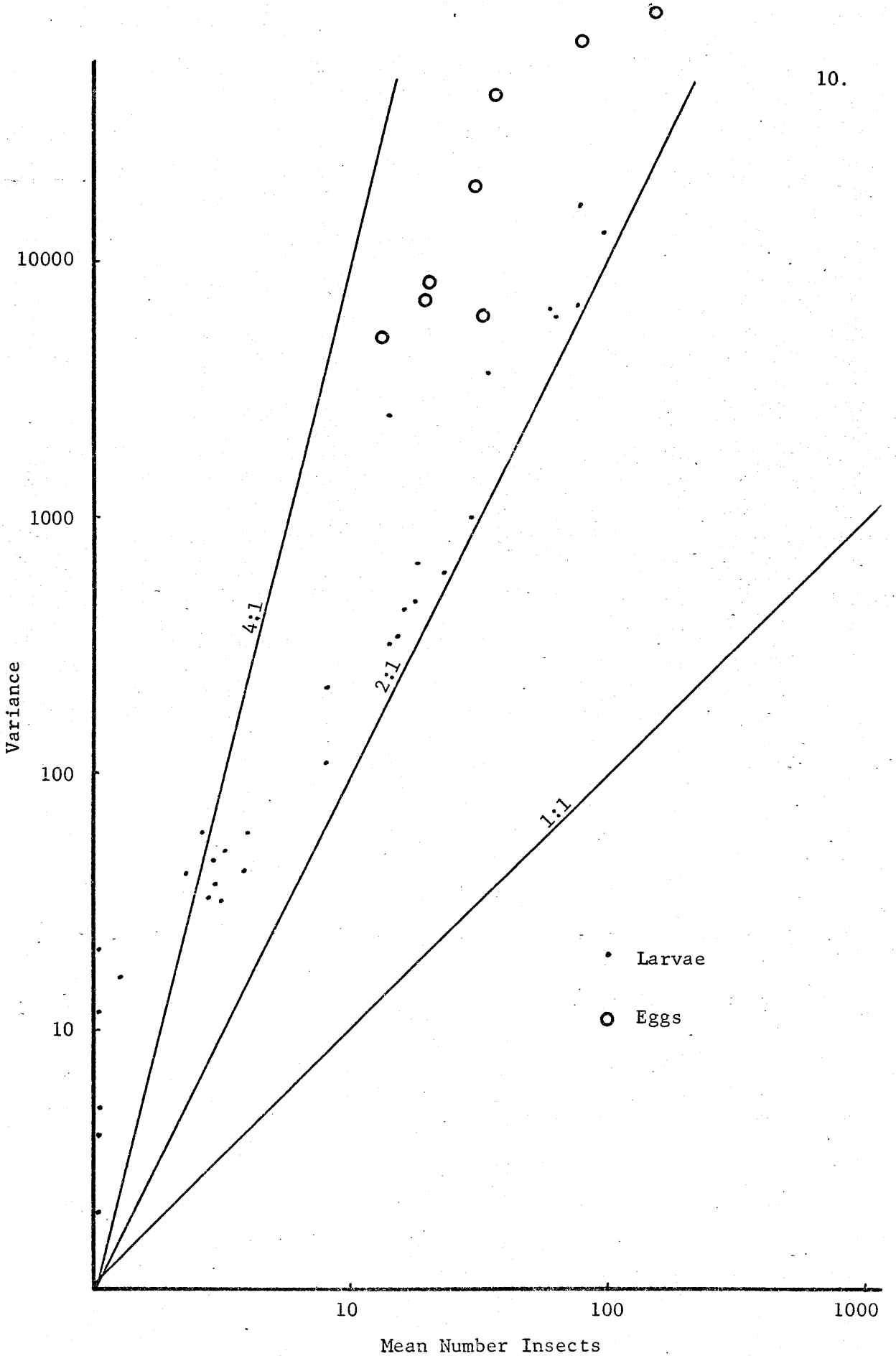

Figure 7.--Relationship of mean and variance for blocks on a logarithmic scale.

Egg sample data were analyzed in two ways: as egg masses and as individual eggs. In population analyses, calculations involving individual egg counts are more meaningful than egg masses because they can be compared directly to numbers of newly hatched larvae from the first larval samples. Because of the relative scarcity of masses on branch samples and the clustering of large numbers of eggs in a mass, variance for individual egg data was exceedingly large.

Calculated values of "F" for the anticipated sources of variation during each sample period are shown in Table 2. All sampling universes proved to be significantly different most of the time.

Table 2.--Analysis of variance showing "F" values for variance between blocks of trees, between trees within blocks, between crown levels within trees and between lateral positions within trees

Sample	Source of Variance			
	Blocks	Trees	Vertical levels	Lateral positions
Eggs (masses)	5.18**	1.64*	9.71**	-
Eggs (numbers)	5.35**	1.76**	13.12**	-
1st larval	0.97 NS	4.62**	137.45**	73.98**
2nd larval	11.82**	7.05**	55.50**	52.53**
3rd larval	7.52**	3.82**	37.99**	14.87**
4th larval	6.57**	2.40**	1.10 NS	3.23**

* Significant within 5 percent level.
** Significant within 1 percent level.

Population Densities in Different Blocks

Egg and larval populations were consistently different at different block locations. Only the first larval sample failed to produce significant differences between blocks, largely because of the high variance associated with vertical levels due to migration of early instar larvae to the tops of trees. Measured densities ranged from less than 1 larva to almost 100 larvae per 1,000 sq.in. of branch area over the course of sampling. Differences between block means were tested further for each sample interval using Duncan's multiple range test. Results are illustrated on a logarithmic scale in Figure 8.

It is encouraging to see in Figure 8 that in the last three sample intervals the same blocks are consistently high (Blocks 1 and 5) or low (Blocks 2 and 3). From the statistical standpoint such consistencies in the data suggest the feasibility of revealing real mortality differences between experimental treatment blocks despite large intra-block variation. Significant differences were also detected between the number of egg masses on separate blocks even though a high error was encountered in egg sampling.

Representativeness in the Tree

Distribution of tree foliage

Since population densities are different among the three vertical levels and two lateral positions in the crown, foliated branch area in these locations must be known so that estimates of insect numbers in each location can be weighted accordingly. When the same number of samples are taken from each crown location, weighting is necessary to estimate a true mean population for the whole tree. If samples are not weighted, the mean will be biased because sampling was not proportional in the crown according to natural foliage distribution. In future studies, when the distribution of foliage is known, samples should be stratified at the outset in such a way that they are representative of foliage area through the tree, thus eliminating the need for weighting later on.

The average distribution of foliage on 15 white fir trees, representative of those trees sampled at Corral Creek, is shown in Table 3. For the purpose at hand, these means adequately describe foliage distribution in the tree. In general foliaged branch area is distributed from top to bottom in a 1:3:1 combination and from inside to outside in a 2:1 ratio, except in the top 1/3 where the amount of outside foliage is equal to that inside. For a truly representative sample, three times as many samples should be collected from the middle crown as from the top or bottom and, except for the top, twice as many samples from the inside of the crown as the outside.

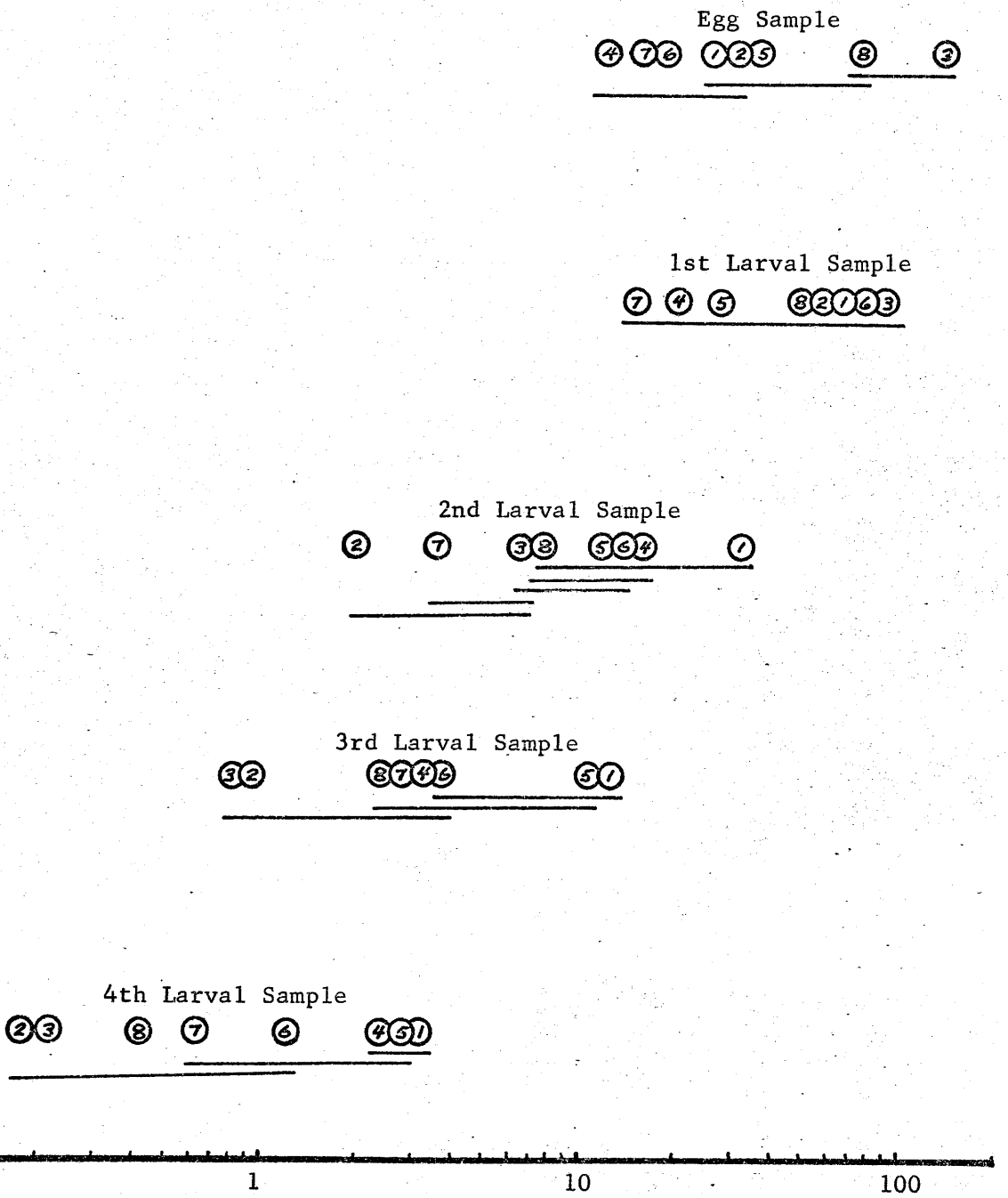
Egg Sample
4 7 6 1 2 5 8 3
1st Larval Sample
7 4 5 8 2 1 6 3
2nd Larval Sample
2 7 3 8 5 6 4 1
3rd Larval Sample
3 2 8 7 4 6 5 1
4th Larval Sample
2 3 8 7 6 4 5 1
.1
1
10
100
Mean Number Insects per 1000 sq. in.

Figure 8.--Mean number of insects on unweighted samples within each block for different sampling periods. Differences between block means using transformed data are tested for significance by Duncan's multiple range test. Means not underscored by the same line are significantly different at the 5 percent level.

Table 3.--Natural distribution of foliage on white fir as a percentage (± one standard error) of the total foliated branch area on the tree. Data are from 15 trees 19 to 33 feet in height and 3.6 to 6.6 inches DBH

Crown level	Outside 17"	Inside	Total
Upper 1/3	11 ± 1.3	10 ± 1.2	21 ± 1.9
Middle 1/3	18 ± 1.2	37 ± 1.9	55 ± 2.7
Lower 1/3	8 ± 1.3	16 ± 2.5	24 ± 3.7
Total	37 ± 1.5	63 ± 1.5	100

Distribution of population densities

Before weighting for foliage distribution, density of insects per unit of branch area was usually significantly different at the various positions sampled in the tree; only the last larval sample, taken when the population was very low, failed to produce a significant difference among vertical levels (Table 2). Eggs were generally concentrated mostly in the lower crown and on the inside branches. In a collection of 71 egg masses from 480 branch samples, only four masses were located on the outer 15 inches of the branch. Subsequent larval samples clearly showed that after hatching a complete turnover in distribution took place; young larvae then migrated upward and outward so that the highest larval densities, in terms of numbers per 1,000 square inches, ultimately occurred in the top outer branches (Figure 9). Larvae were continually found throughout the tree, but in fewer numbers for the same amount of sampled foliage. Differential density patterns in the tree were fairly consistent during larval development despite a drastic reduction in the number of insects. By the fourth sampling period, larvae had become so scarce that sample accuracy suffered considerably resulting in a loss of significance.

Larvae normally cluster at the ends of branches where new needle growth is available which is the favored food, especially in the early instars. Shoot growth is more advanced in the upper and outer branches than in other parts of the tree so migration to these areas is advantageous. Presumably, larvae remain at the top of the tree and at ends of branches because feeding is usually especially favorable at those locations. Because new growth in 1966 was either killed or badly damaged on most sample trees by the late frost, larval concentrations at favored feeding sites may not have been as intense as in normal years. This, of course, would influence distributional patterns recorded in the sampling study and should be kept in mind when interpreting the 1966 results.

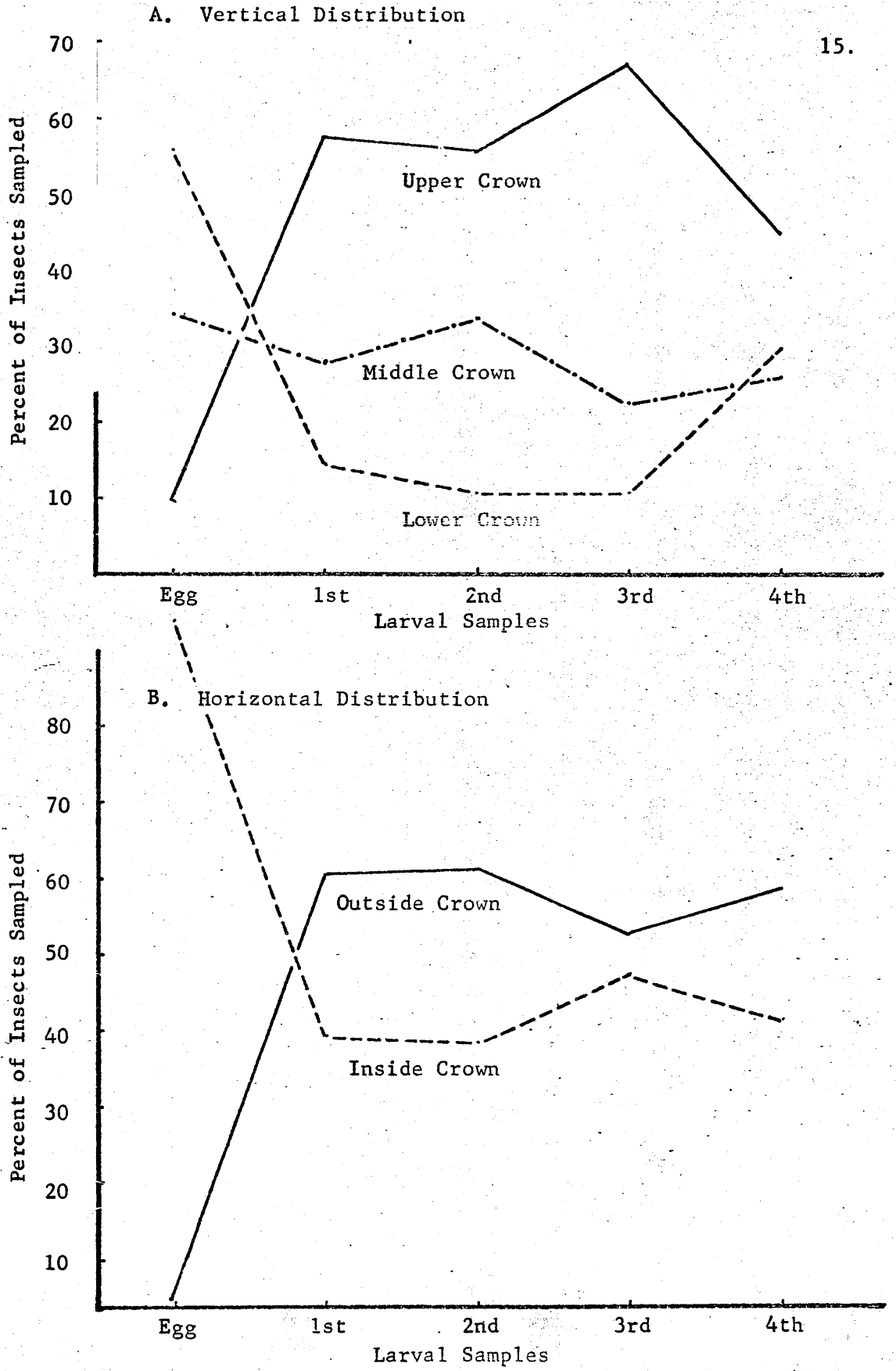

Figure 9.--Distribution of the insect density when the same amount of foliage is sampled in each crown location.

Since sampling in the tree was not representative, mean population densities must be weighted by multiplying the average percentage of insects found in each crown location times the fractional amount of branch area in that location. The sum of the products of the six crown locations is the weighted mean density for the tree. Each product expressed as a percentage of the sum of the six products is the adjusted percentage of all insects in the tree distributed according to the location and amount of branch area. Results of calculations before and after weighting are summarized in Table 4.

Table 4.--Average percentage distribution of insect density in the tree when same amount of foliage is sampled in each crown location (before weighting) and percentage distribution after numbers of insects are weighted according to natural distribution of tree foliage (after weighting)

Crown location	Before weighting				After weighting			
	Total eggs	Larvae			Total eggs	Larvae		
		Outside	Inside	Total		Outside	Inside	Total
Upper 1/3	10	30	26	56	6	23	18	41
Middle 1/3	34	17	11	28	55	20	27	47
Lower 1/3	56	11	5	16	39	6	6	12
Total	100	58	42	100	100	49	51	100

It is interesting to note that although larval density in terms of numbers per unit of branch area is especially concentrated in the top at a combination of approximately 4:2:1, total larval distribution from top to bottom occurs at a 4:5:1 combination. For practical purposes, the total number of larvae on inner branches is actually equivalent to that on the outer 17 inches of foliage since almost twice as much branch area occurs inside as on the outside of the crown.

In the trees sampled, almost all egg masses occurred below the upper crown and the majority of these were in the middle crown where there was most foliage. Since whole branches can be sampled for eggs, it is not necessary to separate egg distribution by outside and inside foliage. From top to bottom, the total number of eggs occurred in a combination of about 1:11:7. Females normally pupate in shaded or sheltered places on the tree so egg masses were usually found in the lower half and on the inside of most crowns. Degree of previous defoliation affects the site of pupation by increasing exposure; as a result masses are probably even lower on heavily defoliated trees than on the lightly defoliated trees sampled mostly in this study.

Representative sampling

When populations are highly variable within the tree each section that contributes significantly to variance should be sampled proportionately to its weight. An exception to this rule is when one section of the tree harbors a population that is indicative of the rest of the tree and from which the population over the whole tree can be accurately estimated. Sampling one section of the tree that is truly representative would have many time-saving advantages, particularly if that section is especially accessible. In this study, the lower crown was usually accessible from the ground. The mid-crown needs to be sampled with an aerial ladder, but it has the potential of being most representative for larval counts because of its position in the tree between high and low population densities.

As a means of evaluating the possibilities of attaining a representative larval sample from a single crown level, block means using data from the lower crown only and the middle crown only were each compared with block means of all three crown levels. Regression and correlation coefficients were calculated to determine how representative each level was of the whole tree if only a single level were sampled. Analyses were made using means not weighted for foliage distribution.

Regressions are shown in Figures 10 and 11. Means from both the lower and mid-crown samples were closely related to means from the whole tree. However, mid-crown samples were superior to lower crown samples since they accounted for more variation (95% vs 79%) and had a smaller standard deviation (6.15 vs 12.09). Moreover, mid-crown means are almost equivalent to whole tree means as indicated by the regression line which is almost at 45 degrees. This was not unexpected as already mentioned because of the central location of the mid-crown foliage.

These data suggest that larval populations estimated from the mid-crown only would satisfactorily represent larvae on the whole tree, at least for the general range of data observed here (5 to 65 larvae per 1,000 square inches). The middle one-third of the tree bole also supports 55 percent of the tree's foliage (Table 3) so is, thus, the most prominent crown component and would need to be sampled the heaviest in a representative sampling plan. Since two times as much foliage area is found on the inside of the middle crown as on the outside, a sampling plan using only that level would require twice as many samples to be taken from the inside as the outside.

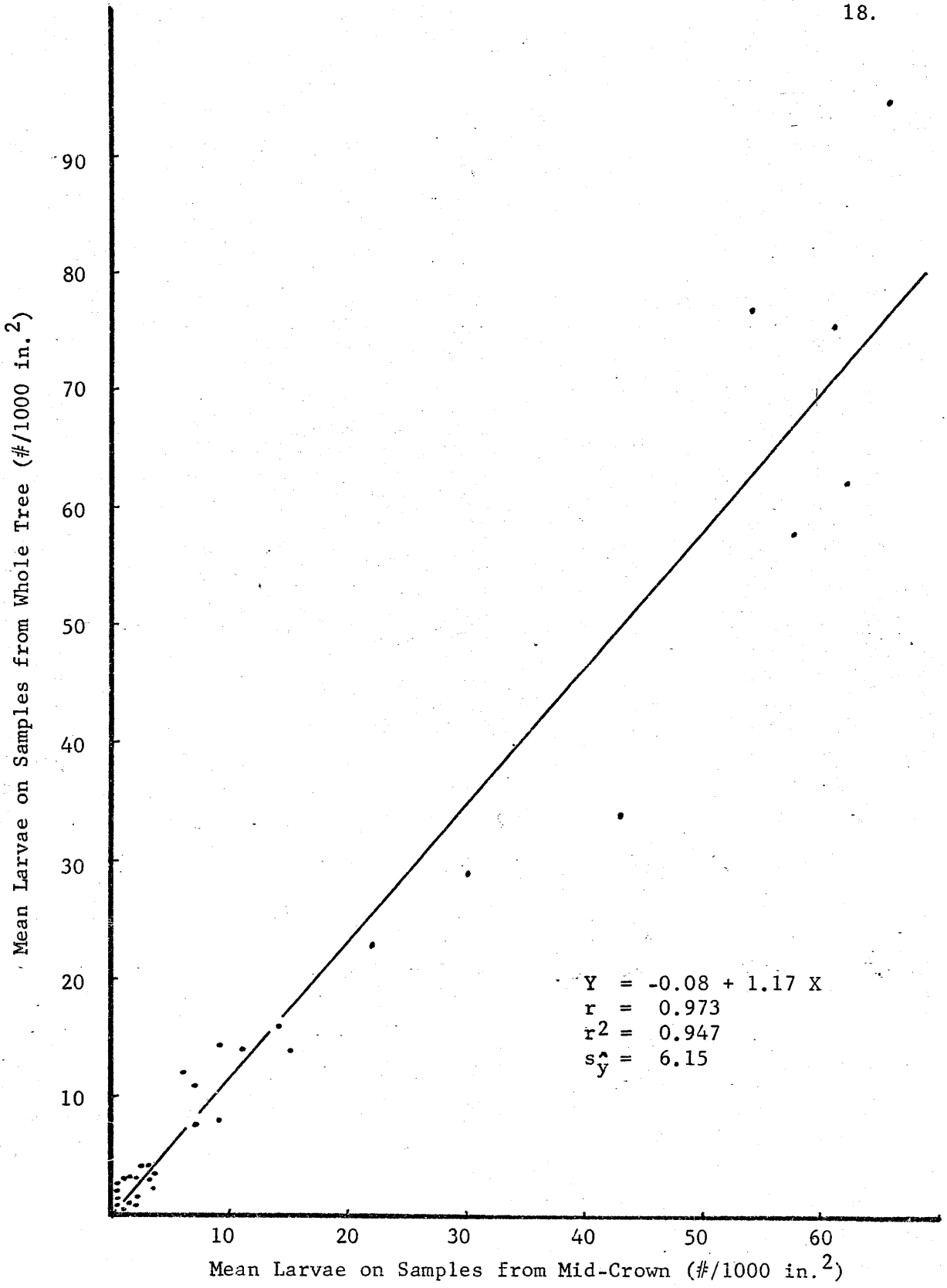

Figure 10.--Regression of mean larvae on samples from whole tree over mean larvae on mid-crown samples.

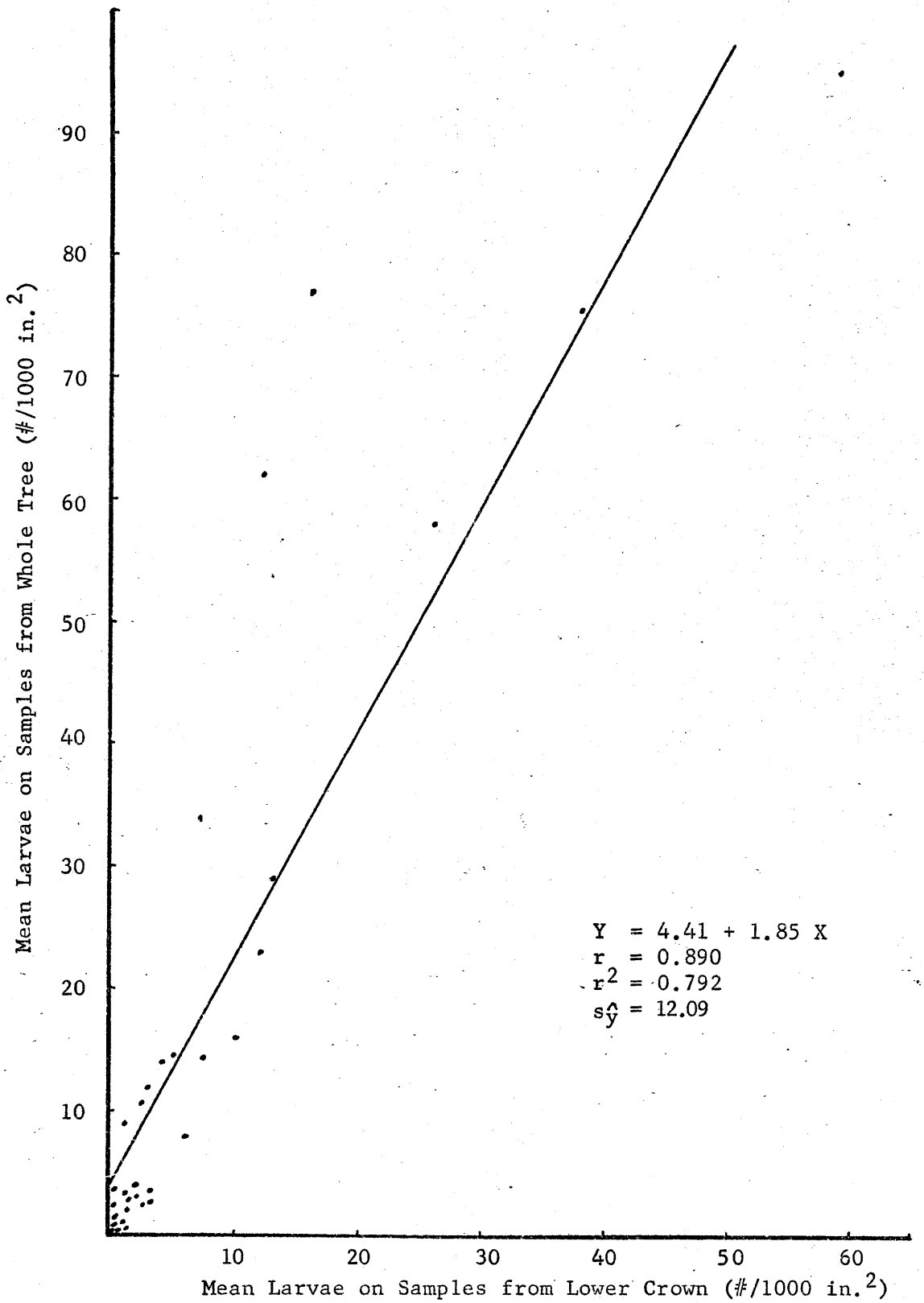

Figure 11.--Regression of mean larvae on samples from whole tree over mean larvae on lower crown samples.

Precision of Estimate

Sampling from whole crown

The precision with which populations are estimated by sampling three crown levels can be determined by calculating the expected standard error for a range of means. When total variance for each block measurement is plotted over its mean a strong relationship between the two is obvious for both egg and larval data (Figures 12 and 13).

The trend of the points eliminates the possibility of a normal distribution. Curves drawn in Figures 12 and 13 are not fitted mathematically to the data, but represent the relationship expected between variance and mean in a negative binomial distribution (Morris, 1955). In this relationship $s^2 = \bar{x} + \bar{x}^2/k$ where k, the coefficient of aggregation, is estimated by $k = \Sigma\bar{x}^2 / (\Sigma s^2 - \Sigma\bar{x})$. The curves seem to fit the plotted points fairly well, especially for means over 10, and should provide an adequate estimate of variance for the range of means likely to be encountered in a tussock moth outbreak.

If data are transformed by $y = \log (x + 1)$ to stabilize variance, the transformation is suitable for larval means over 0.4 on the log scale (approximately 4.0 larvae on the original scale). Figure 14 shows, that for all egg data and larval means below 0.4, variance seems to be related to the mean. Logarithmic transformation is, therefore, apparently ineffective for either egg data or small numbers of larvae. Appropriate transformations for these types of data are needed for standard statistical tests and for determining an independent variance component.

Standard errors were calculated for larvae on both the original and transformed scales and for eggs on only the original scale. These are plotted in Figure 15 as a percent of the mean when sampling was from three crown levels. Precision of estimate is somewhat lower for transformed larval data than for original data which implies that the logarithmic transformation tended to "over-correct." Morris (1955) claims that this occurs when transformation reduces variance disproportionately at the higher mean levels and that the best estimate of variance then lies between the two lines. Degree of precision for egg data is considerably less than for larval data because of the high aggregation associated with egg masses. Accuracy here could possibly be improved somewhat with an effective transformation.

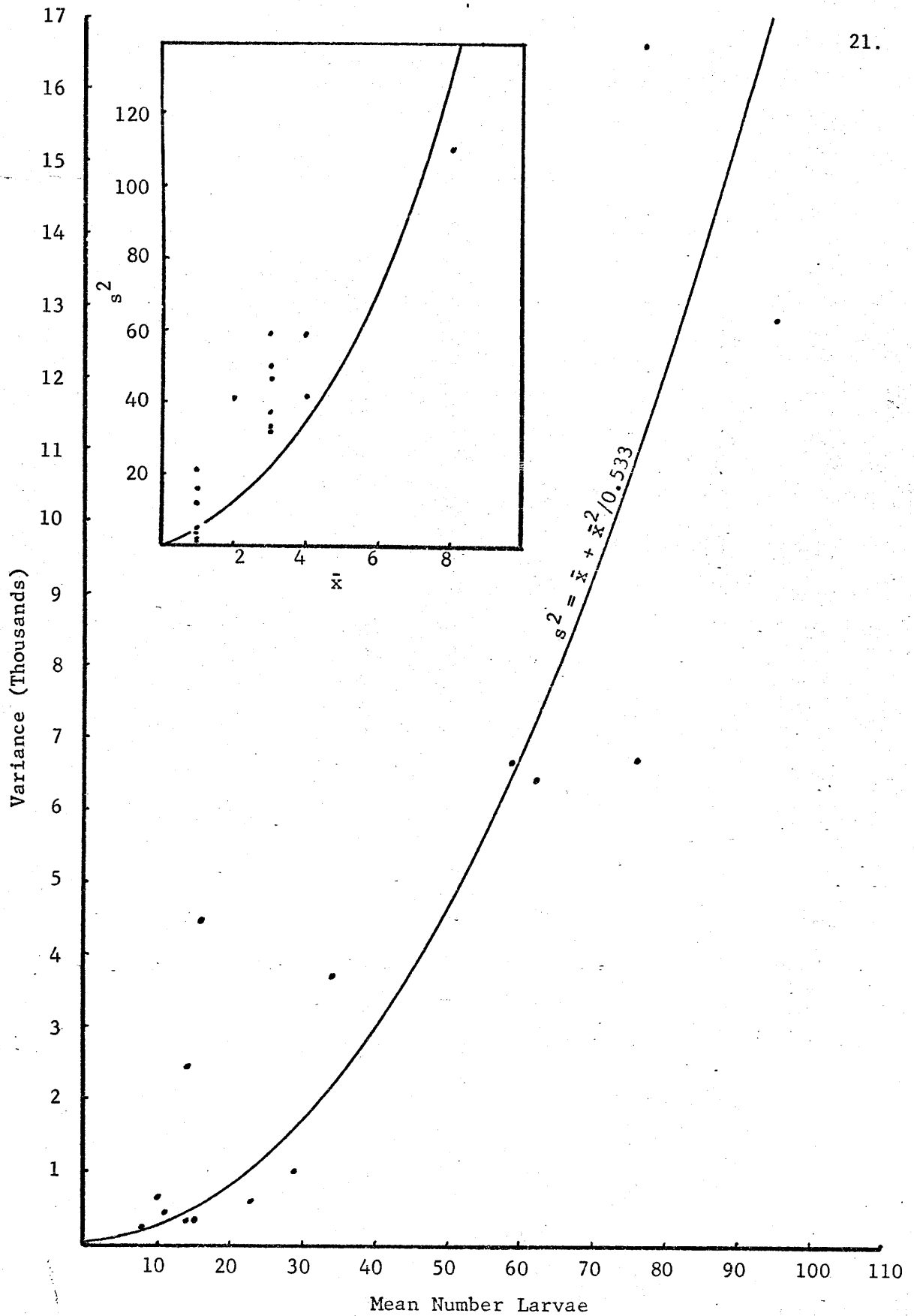

Figure 12.--Relation between intra-block variance and mean number of larvae. Each point represents 60 branch samples taken from 3 crown levels from 10 trees.

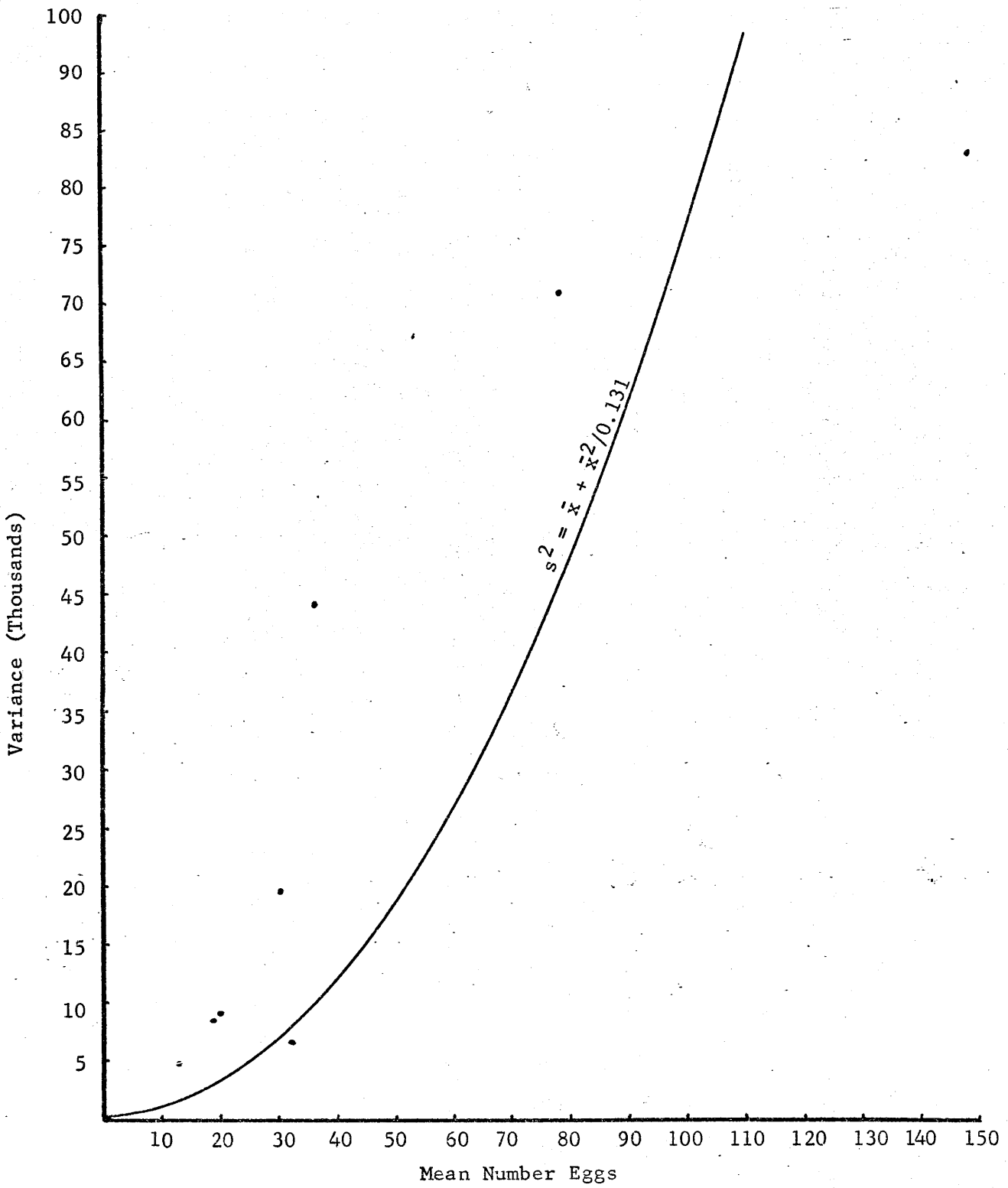

Figure 13.--Relation between intra-block variance and mean number of eggs. Each point represents 60 branch samples from 3 crown levels from 10 trees.

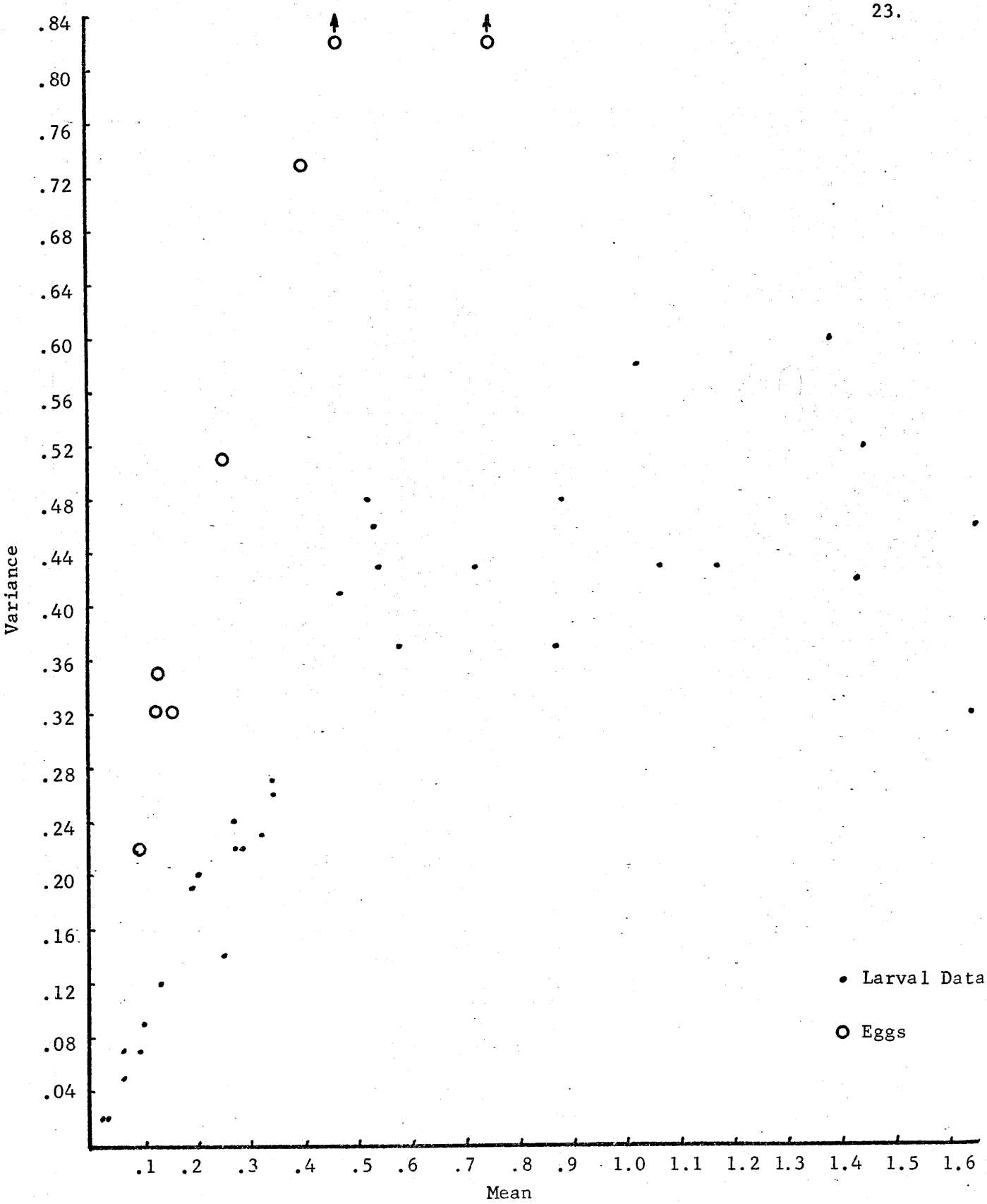

Figure 14.--Comparison of mean and variance of transformed values. Each point represents 6 samples from all crown levels from each of 10 trees.

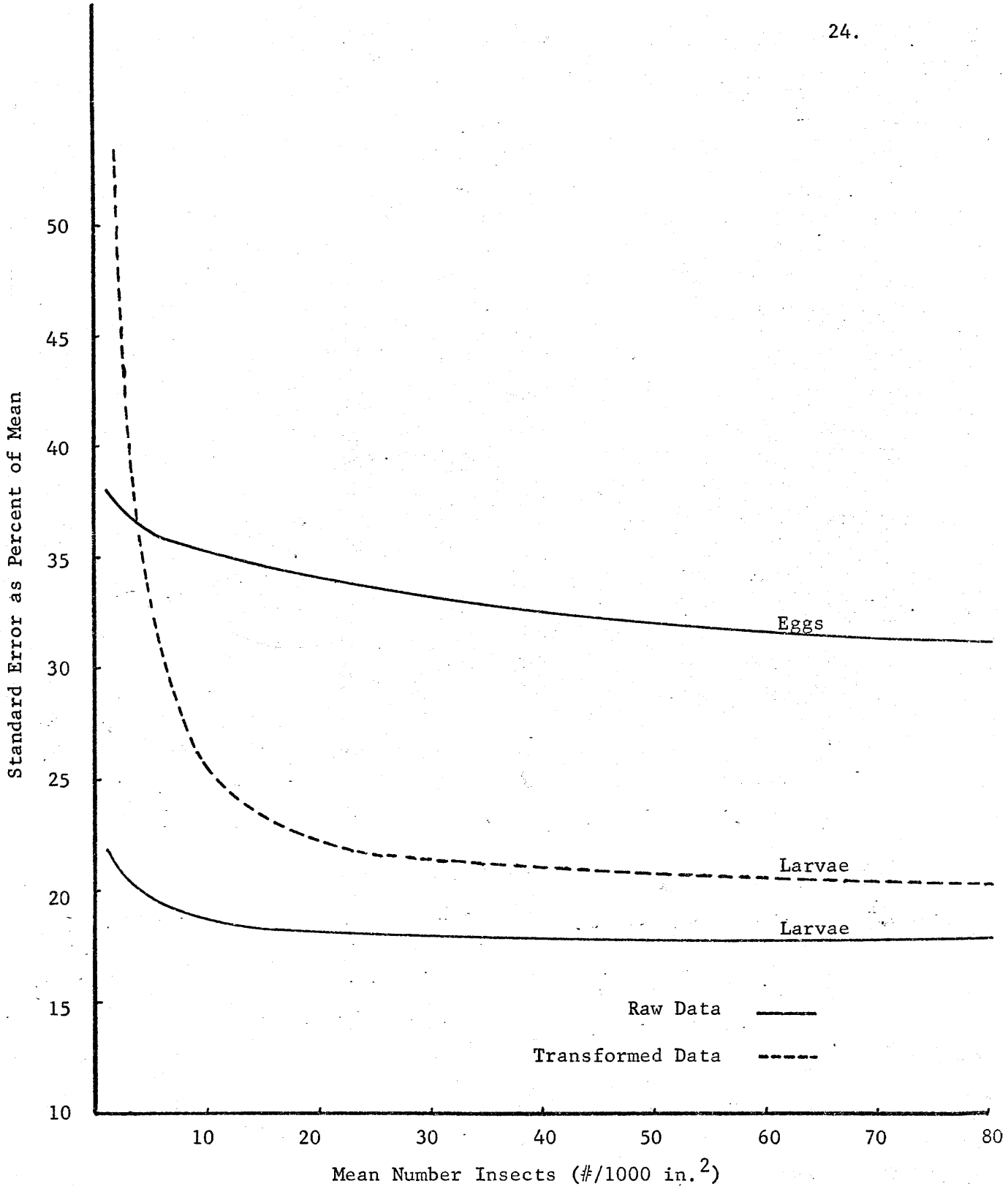

Figure 15.--Degree of precision with which larval and egg populations are defined by sampling 6 branches from 3 crown levels from 10 trees in a block.

Sampling from mid-crown

Mid-crown means for larvae seemed to be representative of whole tree populations and, thus, would be a logical part of the tree on which to concentrate sampling for this stage. Similar to samples from the whole tree the variance of mid-crown larval samples is also strongly related to the mean (Figure 16). Moreover, the curve for a negative binomial distribution describes the data fairly well, especially for means over 10. The calculated coefficient of aggregation, k, is larger for the mid-crown samples than for all samples, which indicates that aggregation is less in the mid-crown than when samples from other parts of the tree are also included. This is expected since samples in the middle of the tree are more uniform than are samples taken from all three crown levels. Consequently, variance is also less among samples from the middle crown than when other levels are included which is further reason for favoring the middle crown as the level for concentrating sampling.

Logarithmic transformation of mid-crown data effectively stabilized variance for the larger means, but again was not an efficient transformation of the smaller means. The appearance of the data in Figure 17 almost suggests a curvilinear relationship with variance lowest for extremely low or high populations and highest for medium populations.

Degree of precision is compared for logarithmic data and original data in Figure 18 and again the transformation is less accurate than when errors are calculated from the raw data. Since the mid-crown standard errors are based on only 20 samples from each block, they are a larger percent of the mean than when the standard error was based on 60 samples from three crown levels. If the same number of samples were examined in both sampling procedures, then the mid-crown samples would be more precise.

Sampling endemic populations

Continuous population studies involve estimating numbers of insects most of the time that are far below those encountered in outbreaks. Many of the probing analyses into population processes will have to be made in relatively low density situations which may require drastically different procedures of sampling from those which are efficient at high densities. The final two sample periods in this study contained seven blocks with a range of means from 0.18 to 1.28 larvae per 1,000 square inches. The accuracy of these means should be a guide for procedures which may be needed for estimating endemic numbers.

Figure 19 shows the relationship between variance and mean for small means and the precision of estimate of these based on samples from three crown levels. The coefficient of aggregation, "k", is especially small for this group indicating a high degree of aggregation among low populations. As would be expected, the standard error for small means is relatively large, implying that appreciably more samples will need to be examined in endemic situations to attain precision equivalent to that in outbreaks.

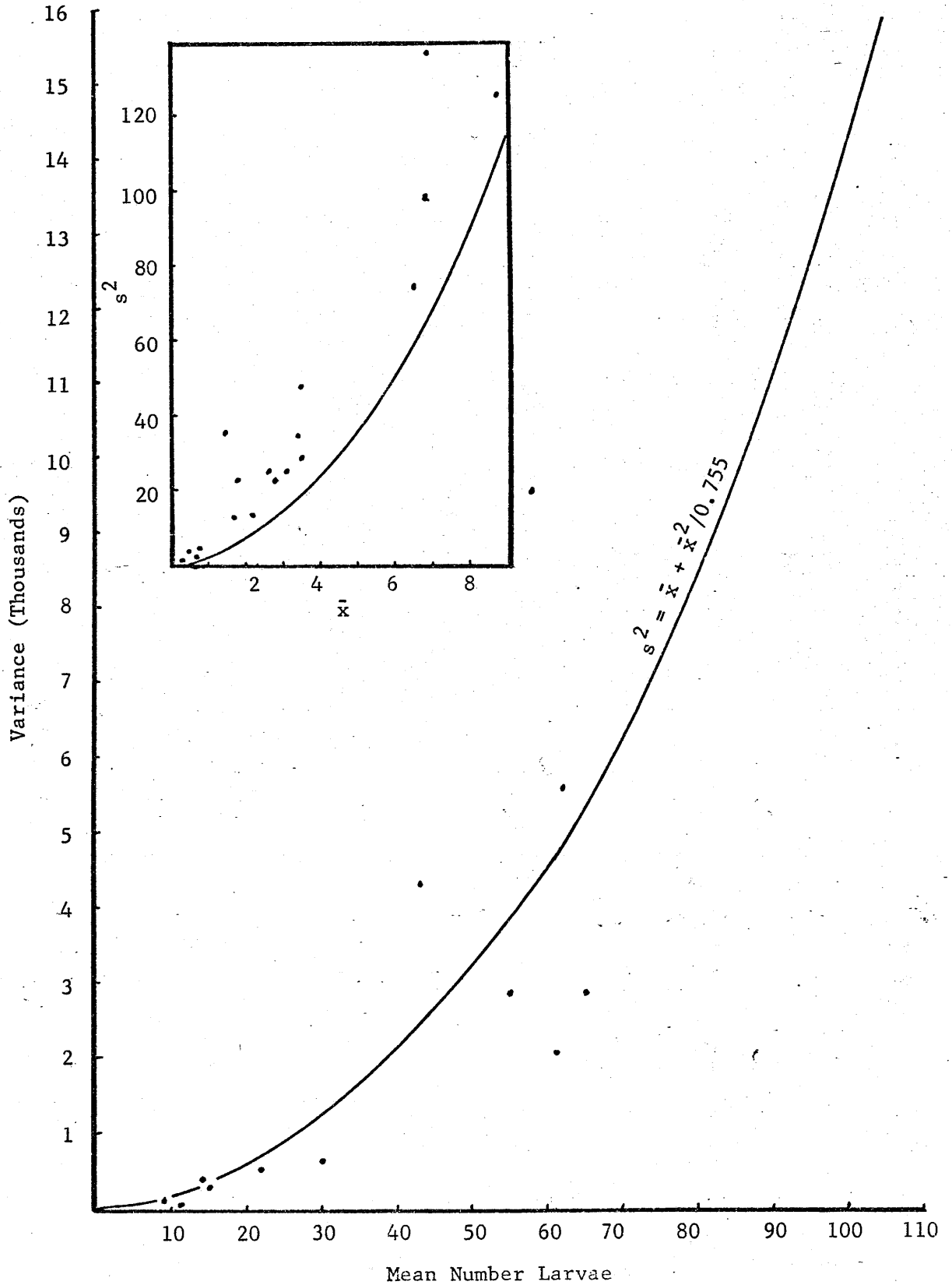

Figure 16.--Relation between intra-block variance and mean number of larvae from mid-crown samples only. Each point represents 20 branch samples taken from mid-crown level from 10 trees.

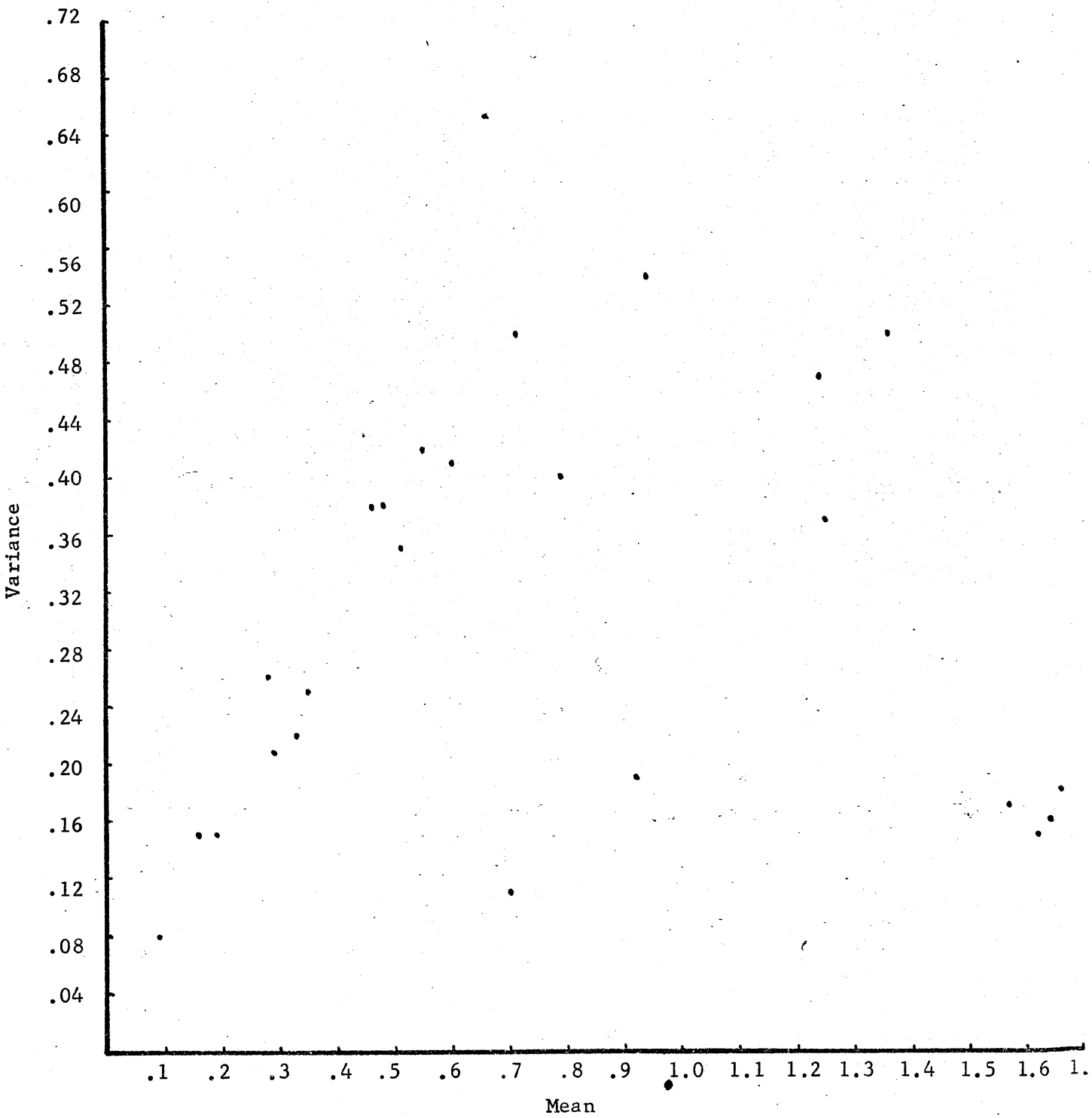

Figure 17.--Comparison of mean and variance of transformed values. Each point represents 2 samples from mid-crown from each of 10 trees in a block.

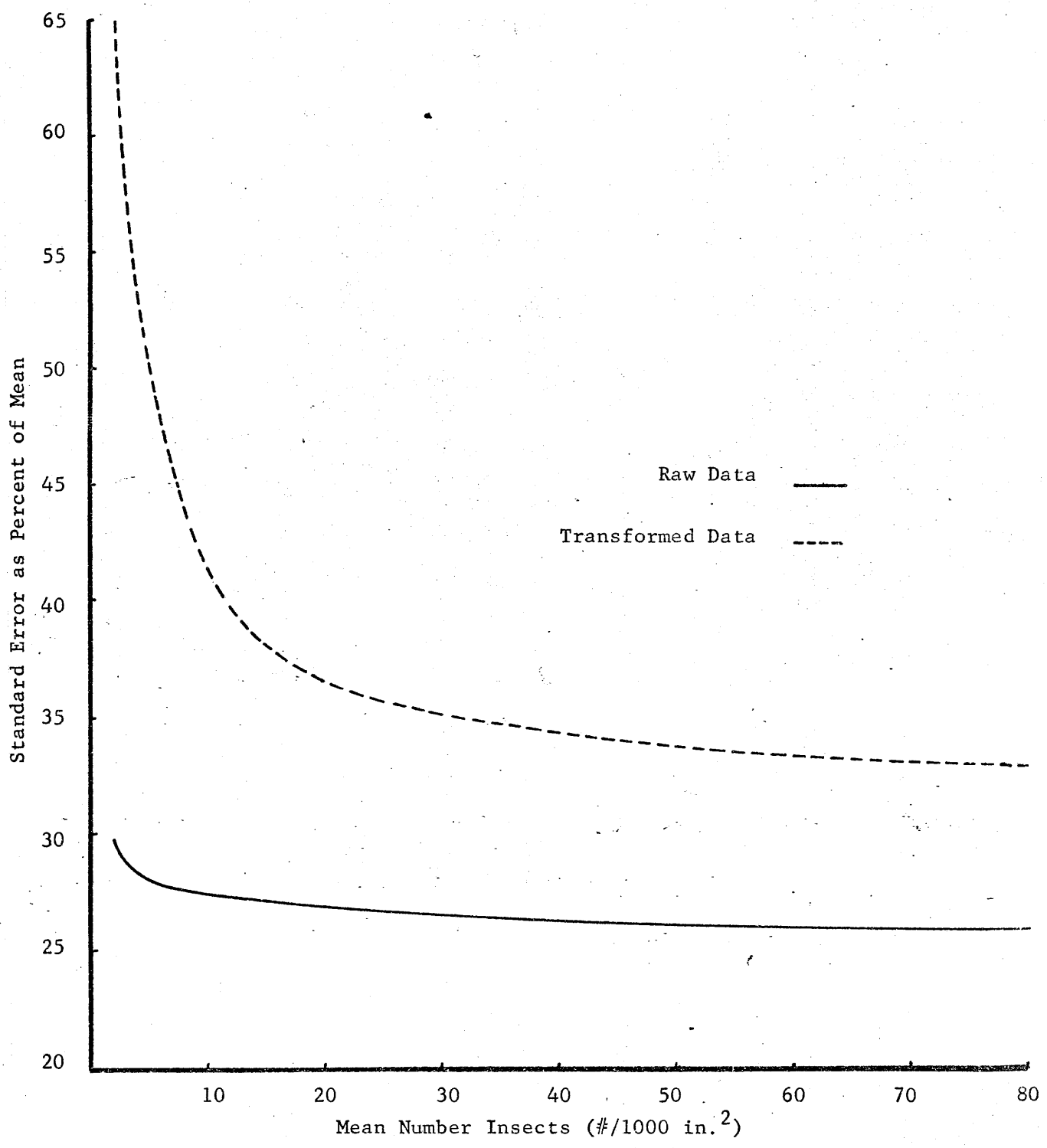

Figure 18.--Degree of precision with which larval population is defined by sampling 2 branches from mid-crown from 10 trees in each block.

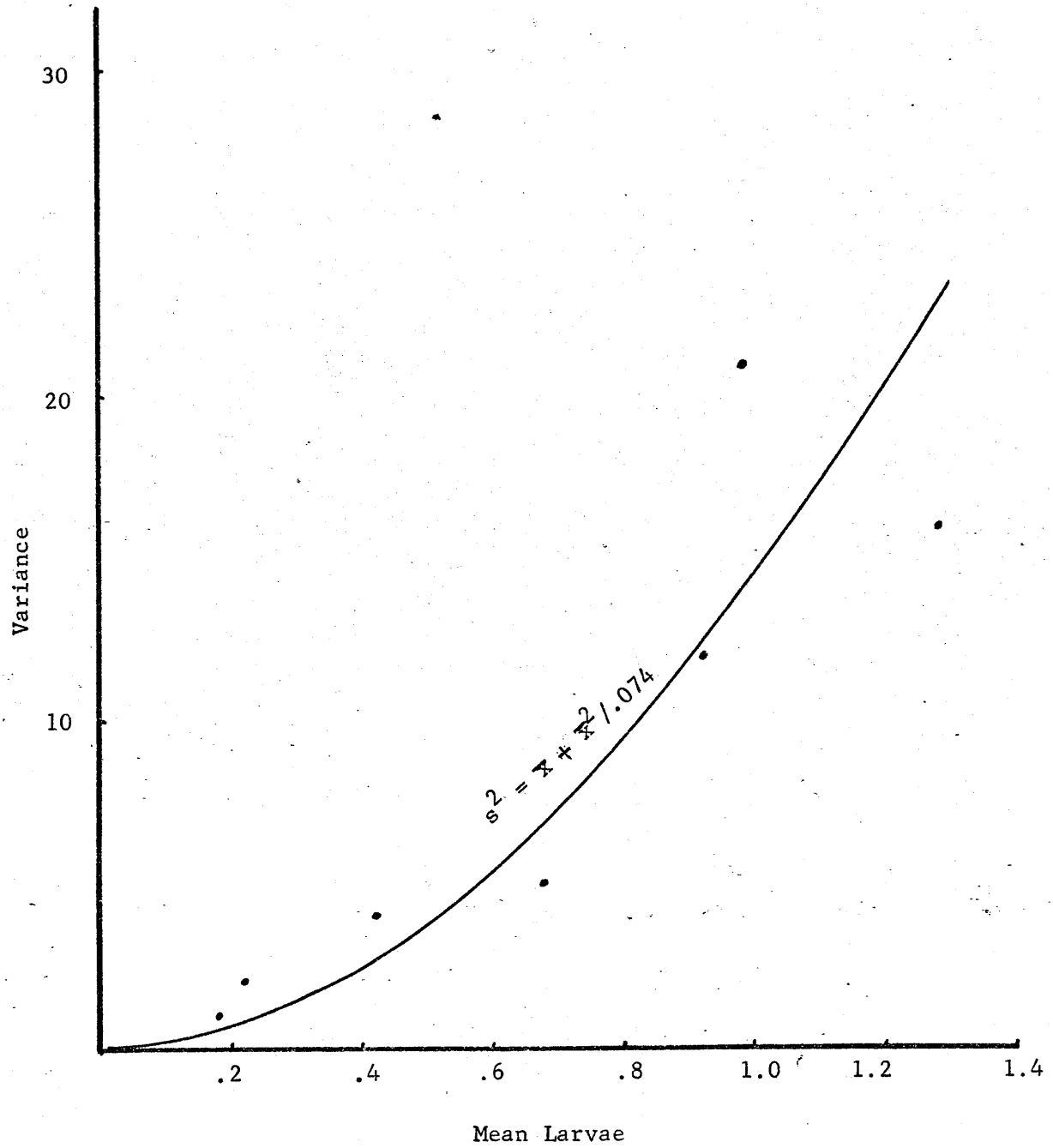

Figure 19.--Comparison of mean and variance for endemic populations.

Optimum Sample Size

A prime consideration in developing sampling methods is the number of units needed to reach a given degree of precision. This, of course, brings up the question of how much precision is needed in the estimates for the population analyses that will follow. Morris (1955) reviewed this question, but reached no definite conclusion primarily because of a general lack of data from sampling studies. For his calculations with the budworm he set a required standard error of 10 percent of the mean, but added that this was purely arbitrary. Data from population studies of other defoliators, however, have shown that significant analyses are possible when standard errors are considerably higher than 10 percent (Embree, 1965; Klomp, 1966). Results of preliminary analyses in this study also indicate that standard errors of 15 to 20 percent of the mean may be adequate for tussock moth data. About four times as much sampling is usually needed to reduce the error from 20 percent to 10 percent so that the added effort for increased precision in this case would probably not justify the results. For this reason, the following calculations for sample size are based on a standard error of 15 percent or 20 percent of the mean.

Number of sample units per block

Total sample units required for a given degree of precision on each block were calculated by $n = (s/s_{\bar{x}})^2$ where variance was taken from the relationship shown in Figures 12, 13, and 16. The results plotted on a logarithmic scale are shown in Figure 20. From 3 to 4 times as many samples of eggs are necessary to reach the same precision as for larvae. Because of less heterogeneity of insect numbers in the mid-crown, fewer total samples are needed from that level than when all crown levels are examined.

Number of sample units per tree

The question arises as to how many samples should be taken from each tree for the most efficient sampling procedure. This is a problem of the relationship between the components of variance contributed by individual sample units and by trees. Since transformation was not effective in stabilizing variance in blocks with a low mean, data from the final larval sample were not included in the variance component analysis which requires a common variance not related to the mean. When larval data were analyzed by blocks the average of the mean squares between trees was larger than the average of mean squares within trees, but the difference was significant for only 7 of the 24 blocks examined. Nevertheless, this permitted an analysis using the components of variance technique since the variance component due to trees is estimated by

$$s_t^2 = \frac{\text{Mean square trees - Mean square samples}}{\text{Number samples per tree}} \quad \ldots\ldots (2)$$

(Snedecor, 1946; Morris and Reeks, 1954). For egg data the average of mean square between trees was not larger than the average within trees which prohibited any calculation of a variance component.

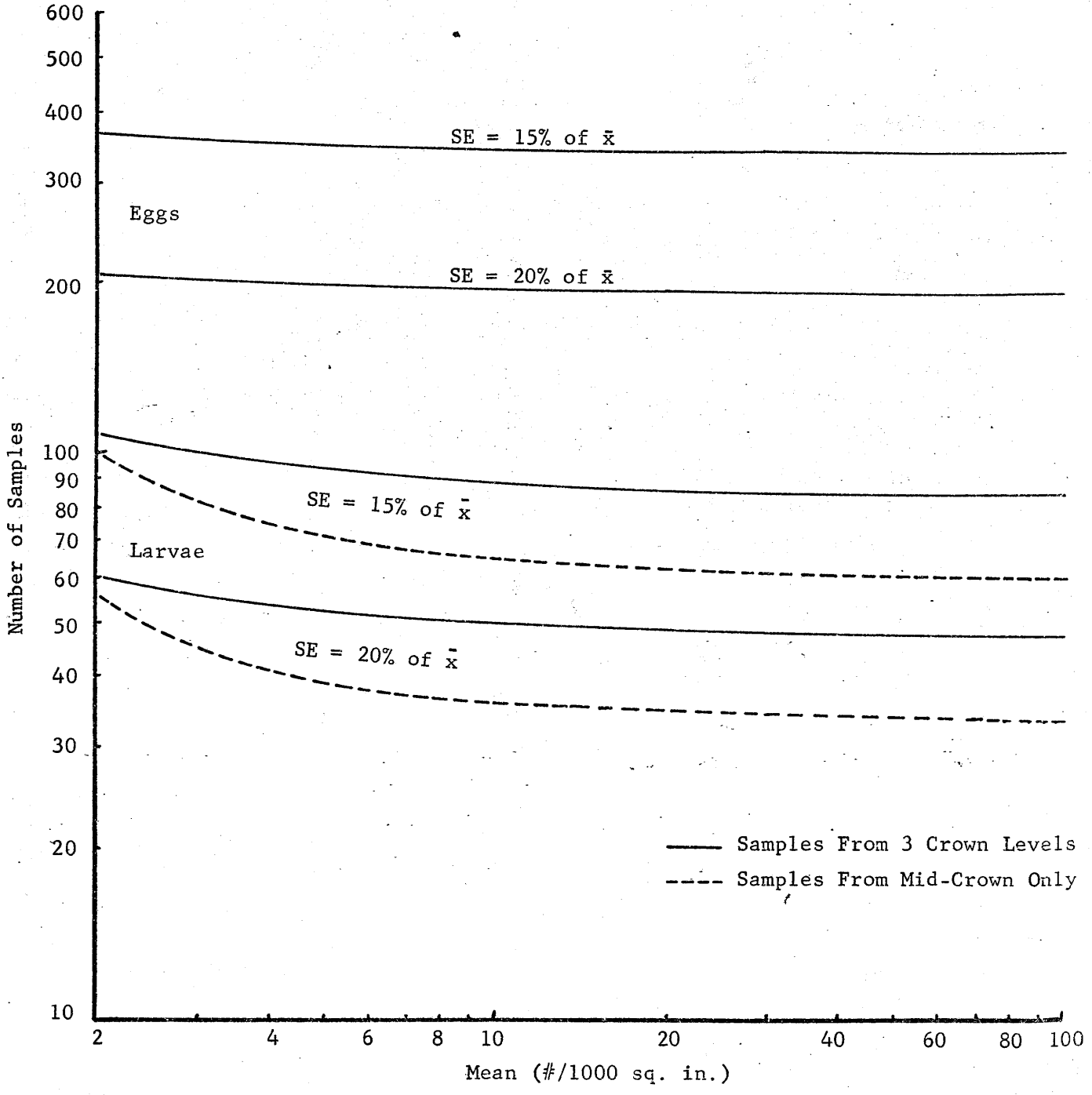

Figure 20.--Number of samples required per block for different degrees of precision.

In the statistical sense both larval and egg data imply that average mean squares for trees and for samples within trees are estimates of the same variance since "F" is not usually significantly different from one. However, since almost a third of the blocks from which larval data were collected produced a significant "F" it seems probable that the average mean squares for trees may be somewhat larger than that for samples. If this is true, an estimate of the most efficient combination of samples and trees can be calculated using the formula

$$N_t = \frac{N_s\, s_t^2 + s_s^2}{s_{\bar{x}}^2\, N_s} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

where N_t is number of trees, N_s is number of samples per tree, s_t^2 is mean square for trees and s_s^2 is mean square for samples within trees, both expressed as a component of variance, and $s_{\bar{x}}^2$ is the desired standard error squared (Le Clerg et al, 1962; Morris, 1955). Using transformed data the best estimates of variance when sampling larvae from the whole tree or just the middle crown are

	Whole tree	Mid-crown
Intertree variance ($s_s^2 + s_t^2$)	.357	.293
s_s^2	.339	.269
s_t^2	.018	.024

Standard errors were calculated using the system of Morris (1955) so that they correspond to 20 percent of the mean on the original scale.

Various combinations of branch samples and trees which will give equal sampling precision for a range of expected population levels are presented in Table 5. The total number of samples required by this calculation are larger than the number calculated using original data before transformation (Figure 20). This was expected since the logarithmic transformation apparently over-corrected in stabilizing variance which resulted in a higher standard error when using transformed data. Therefore, the sample sizes shown in Table 5 should produce means well within the range of a 20 percent error. If only the mid-crown is sampled, fewer total units will need to be examined than if the whole tree is sampled.

Since foliage is distributed in the crown disproportionately, samples need to be arranged so that they are representative. If the whole tree is to be examined, this would mean taking three times as many samples from the middle as from either the top or bottom and more in the middle of the tree than the outside. A representative sampling scheme utilizing each crown level would, therefore, require a fairly large number of samples per tree which would reduce the total trees needed, but tend to increase the total number of units that have to be examined. Since moving sampling equipment from tree to tree in a heavy stand is not

Table 5.--Combinations of branch samples per tree N_s and trees per 1-acre block N_t that will provide equal sampling precision of larvae when standard error is set at 20 percent of the mean. (Data transformed by y = log (S + 1))

N_s	$\bar{x}$ = 5		$\bar{x}$ = 10		$\bar{x}$ = 20		$\bar{x}$ = 40		$\bar{x}$ = 80	
	N_t	N_sN_t	N_t	N_sN_t	N_t	N_sN_t	N_t	N_sN_t	N_t	N_sN_t
	All crown levels									
1	127	127	89	89	72	72	64	64	61	61
2	66	132	47	94	38	76	34	68	31	62
3	46	138	33	99	26	78	24	72	22	66
4	36	144	26	104	21	84	19	76	17	68
5	30	150	21	105	17	85	15	75	15	75
6	26	156	19	114	15	90	13	78	13	78
	Mid-crown levels									
1	104	104	73	73	59	59	53	53	50	50
2	56	112	40	80	32	64	28	56	27	54
3	40	120	28	84	23	69	20	60	19	57
4	32	128	23	92	18	72	16	64	16	64
5	28	140	19	95	16	80	14	70	13	65
6	24	144	17	102	14	84	12	72	12	72

particularly a difficult problem, the biggest chance for improvement probably lies with reducing the total number of samples that have to be examined. Moreover, removing a large number of samples from a single tree several times during the summer has another disadvantage of depleting the tree of foliage quicker than if a small number were taken at each sample period. This could be a problem, especially in the tops of trees and in studies where the same trees must be examined for several years.

As already described, larval populations in the middle crown seem to be remarkably representative of the whole tree. Foliage in the mid-crown is distributed from the inside to outside in a 2:1 combination so that a total of these samples, two from the inside crown and one from the outside crown, would adequately represent this level. Also, the middle crown contains about 60 percent of the foliage in the tree which would not easily be depleted by continuous sampling.

According to Table 5, from 57 to 120 samples per block would be needed over the range of means given when 3 samples are taken from each tree. If we can assume that transformation over-corrected the data, then the best estimate of sample size probably lies below these figures, but above those shown in Figure 20. In this case, from 45 to 78 samples would be halfway between the two estimates for the same range of means. These samples would need to be collected respectively from 15 to 26 trees in each block where a population measurement was desired. The total number of units needed is similar to that examined on each block in this study except that sample size is now adjusted for expected mean and is distributed to sample efficiently the known sources of variance. The result is that more trees can now be examined which is desirable to gain a thorough representation in the block; this can apparently be done without increasing the total number of units to be examined or affecting the estimate of the true mean.

Number of sample units per infestation

An entire infestation has a wider range of population levels among trees than a block which in comparison is a relatively homogenous unit. Therefore, if only one mean is to be estimated for the outbreak, the required sample size will be different than for a one-acre block. When samples were pooled from the whole infestation, intertree variance was significantly greater than intratree variance for each of the larval sampling periods. Using whole tree data from the first three larval samples variance components were estimated as follows:

Intertree variance ($s_s^2 + s_t^2$)	.367
s_s^2	.263
s_t^2	.104

As expected, the variance component due to trees is considerably larger for pooled data from the overall outbreak than for individual blocks. By substituting these values in formula (3), equivalent combinations of samples were determined for the whole infestation (Table 6).

Sample sizes shown in Table 6 are larger than those in Table 5a because of the larger variance component associated with trees. Similarly, as more samples are taken from a tree the total number of samples needed rises sharply, since the required number of trees cannot be reduced proportionately to the increase in samples from a tree. As in the previous calculation of sample size, these are maximum figures needed since transformation tended to increase variance somewhat, especially among the smaller means. Judging from results in Table 5, mid-crown sampling would reduce sample size estimates in Table 6 by about 15 percent because of a lower variance in that crown region. Trees used for sampling should be randomly located throughout the area of infestation for which a population estimate is desired.

Number of sample units for endemic populations

Low means have a much smaller degree of precision when compared with large means estimated from the same number of samples. Likewise, the same precision may not be required of endemic populations as for outbreaks depending on the use to which the data are to be put. In rigid analyses of population processes, particularly when all means are small, a standard error within 20 percent of the mean will probably be needed for low as well as high populations.

Using variances determined from the curve in Figure 19, sample size was calculated for larval means of 1.2 or less. Figure 21 shows these results. The number of samples needed to estimate an endemic population within 20 percent of its mean exceeds by several times the number required for large populations. Since endemic populations have never been estimated, the expected range in which these may occur is not known. It is quite likely that extremely low numbers will need to be estimated or studied by an entirely different technique.

Table 6.--Combinations of branch samples per tree (N_s) and trees per infestation(N_t) that will provide equal sampling precision when standard error is set at 20 percent of the mean. (Data transformed by y = log (x + 1))

N_s	$\bar{x}$ = 5		$\bar{x}$ = 10		$\bar{x}$ = 20		$\bar{x}$ = 40		$\bar{x}$ = 80	
	N_t	N_sN_t	N_t	N_sN_t	N_t	N_sN_t	N_t	N_sN_t	N_t	N_sN_t
1	130	130	91	91	74	74	66	66	63	63
2	84	168	59	118	47	94	42	84	40	80
3	68	204	48	144	39	117	35	105	33	99
4	60	240	42	168	34	136	31	124	30	120
5	56	280	39	195	32	160	28	140	27	135
6	52	312	37	222	30	180	27	162	25	150

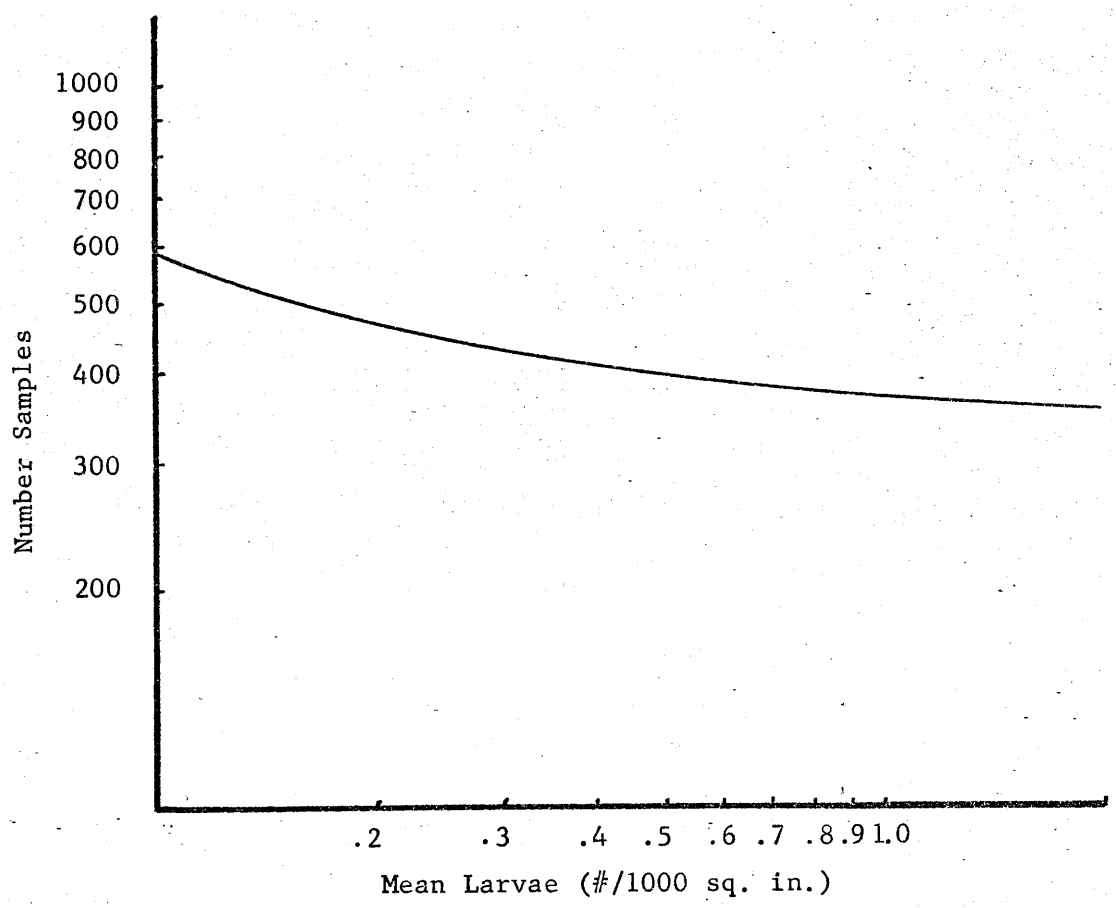

Figure 21.--Number samples required for estimating an endemic population within a 20 percent accuracy.

Absolute Populations

The only true statement of population size must be expressed in terms of a unit which does not vary. Morris (1955) refers to this as the absolute population and states that the only absolute unit for forest insects is the acre. All populations discussed so far have been in terms of average numbers per unit of branch area which can be a highly variable item on any acre. To reach a true value of a population size that can be compared to populations in other areas, the average number of insects per branch area needs to be converted to an acre basis. This requires an estimate of total foliated branch area per acre.

Branch area was measured on fifteen felled white firs and then related to square inches of basal area of each tree. The regression of branch area on basal area for this sample was highly significant although there was considerable variability among some trees of the same size (Figure 22). Position of a tree in the stand has a marked effect on its total branch area. Open-grown trees usually have a perfectly symmetrical arrangement of branch foliage while trees in a cluster may be almost devoid of branches on the crowded side. These growth features should perhaps be considered where branch area is being determined in either very dense or open stands. Where there is a variety of conditions, a general regression that includes the diameter range of the trees in the stand will probably have to be sufficient.

If it can be assumed that a linear relationship between basal area and foliated branch area actually exists as shown in Figure 22, then total branch area per acre can be estimated by

$$\text{Branch Area (M sq.in./acre)} = 2 \times \text{Basal Area (sq.in./acre)} \quad . \; . \; . \; (4)$$

where diameters in the stand are in the range used to compute the regression.

As an example, a young stand of white fir with 100 sq.ft. of basal area per acre where trees were mostly in the 3 to 7 inch diameter range would be calculated to have $2 \times (100 \times 144) = 28{,}800$ M sq.in. of branch area. If these had an average of 50 larvae per 1,000 sq.in. of branch area, then we would estimate $50 \times 28{,}800 = 1.44$ million larvae per acre. A stand with only 50 sq.ft. of basal area would have to have twice as many larvae per branch unit to have the same absolute population. Where stand conditions are similar throughout the infestation, the estimate of insects per unit of branch area is probably adequate for comparative purposes. If a significant number of insects are found on another host species, such as pine, then this should also be taken into consideration.

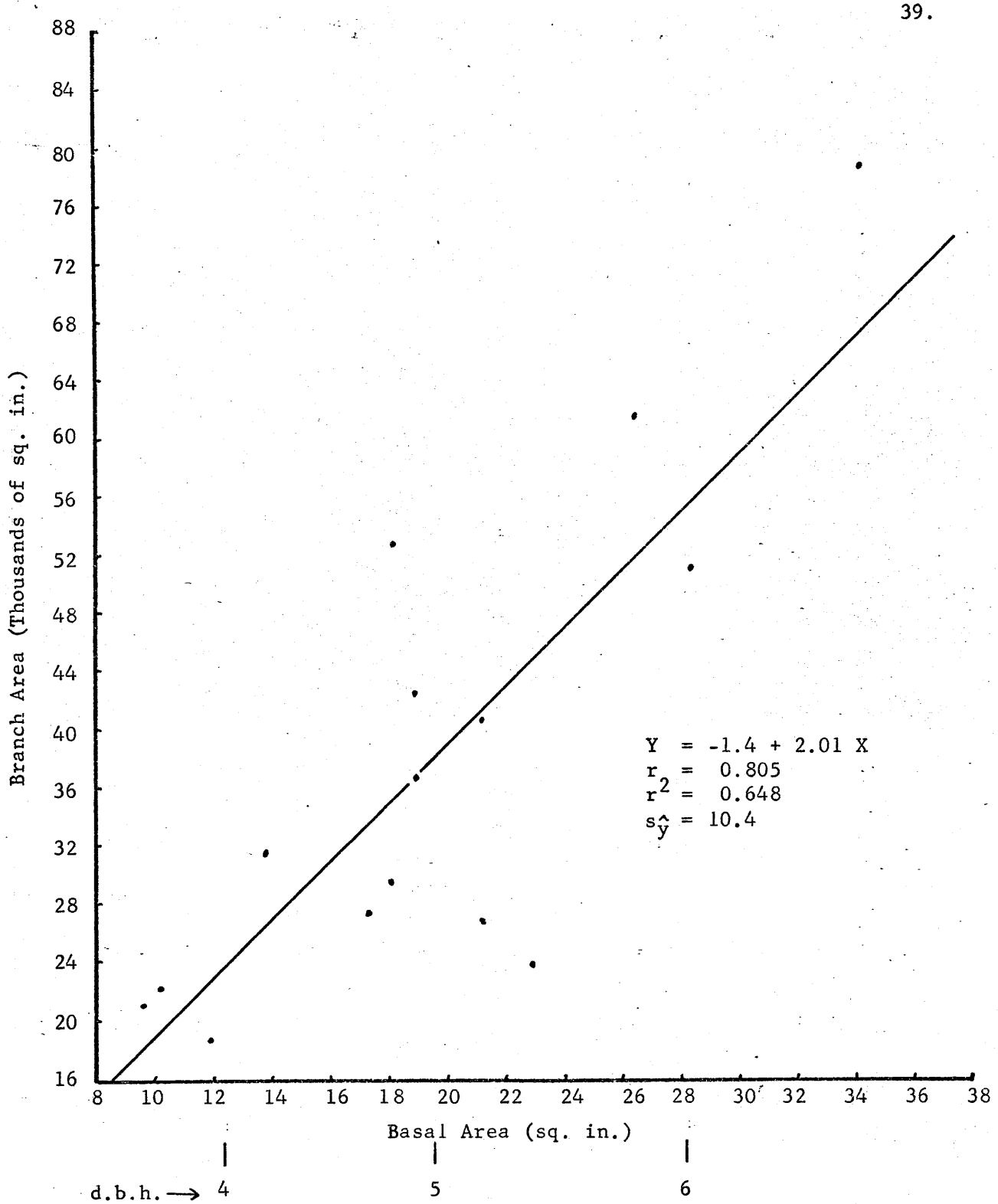

Figure 22.--Regression of total branch area on basal area for white fir.

Sequential Sampling

Tussock moth populations could be categorized into general classification levels by sequential sampling which usually requires much less sampling than is needed for precise population estimates. This system might be useful for rapidly evaluating new outbreaks or for stratifying population levels in going infestations. The method would be especially helpful for egg assessment since tussock moth outbreaks are often first scouted in this stage. Sampling by a sequential plan is a methodical way of examining infestations quickly and yet produces standardized results for all areas visited.

Enough sampling data were collected in this study to provide the essential information needed to calculate a preliminary sequential plan for both eggs and larvae. Frequency distributions of eggs and larvae seemed to be close to the negative binomial. Formulas for calculating decision lines for data in a negative binomial distribution have been published by Waters (1955) and were used for the computations. A pooled estimate of the coefficient, "k", has already been determined for a wide range of means. For eggs k was 0.131 and for larvae in the middle crown it was 0.755. A 90 percent confidence level was used for both alpha and beta errors so there is an equal chance of accepting or rejecting a classification when the alternative is true.

Three population classes have been designated for both eggs and larvae. These were set somewhat close together so that decisions could be reached as quickly as possible. The descriptions light, medium, and heavy are strictly arbitrary and are used simply to describe the various population levels designated. All populations decline sharply through the season so that density classifications always need to be related to the age of the larvae examined.

Light: 5 or less insects per 1,000 sq.in. of branch foliage.

Medium: 12 to 18 insects per 1,000 sq.in. of branch foliage.

Heavy: 25 or more insects per 1,000 sq.in. of branch foliage.

Classification of egg populations

Classification of numbers of eggs with any reliability can be especially difficult because of the large concentration of eggs in a mass. This is reflected in the unusually low value of "k" when eggs are analyzed as individuals and not as egg masses. Viewing eggs as individuals, however, rather than masses is an advantage where comparisons are to be made with larvae in the same area. Therefore, all original analyses were made on actual numbers of eggs after they were separated from the mass. Counts showed that number of eggs per mass averaged 161 ± 9.6. Since only masses can be counted in the field the scale in the sequential graph has been converted back to masses using a factor of 160. Original computations, however, were performed with numbers of eggs as specified in the density classes that were established for the sequential plan.

Should be corrected.

Sequential graphs that define the decision lines for sampling eggs are shown in Figure 23. The horizontal scale is number of trees when five whole branches are examined from each tree in a 1:3:1 combination from top to bottom. Further studies of egg distribution may indicate one part of the tree is representative of the whole tree and, then, reduce the number of branches that need to be examined. Unfortunately, data now available are not adequate to show any such relationship; therefore, egg sampling must be representative of all crown levels.

2:2:1 ?

Numerical values from Figure 23 are listed in Table 7 where the progress of sampling can be followed more easily. The plan stipulates that at least 5 trees (25 samples) must be examined before an infestation can be classified as "light." However, medium or heavy infestations can be classified sooner which could be a great savings in time where only general classifications are wanted, but by standard procedures.

Classification of larval populations

Larval populations can be sampled from the middle crown only since this area has already been shown to be representative of the whole tree. Moreover, larvae are less aggregated than eggs which reduces considerably the number of trees needed to be examined to reach a decision. The sequential graphs for larvae are shown in Figure 24 and the tabular values in Table 8. Three samples are taken from the mid-crown of each tree, two from the inside and one from the outside. As with eggs, all counted larvae are converted to a common unit of 1,000 square inches of branch area.

Contrary to the relatively large number of trees that must be examined to classify a light egg population, larvae can be classified as "light" after examining as few as two trees (6 samples). This implies that sampling early-instar larvae may be a more efficient means of reconnaissance than sampling eggs which require a considerably larger number of samples. Age of the larvae at the time of the survey also needs to be kept in mind since even rising populations suffer a high attrition rate through the season.

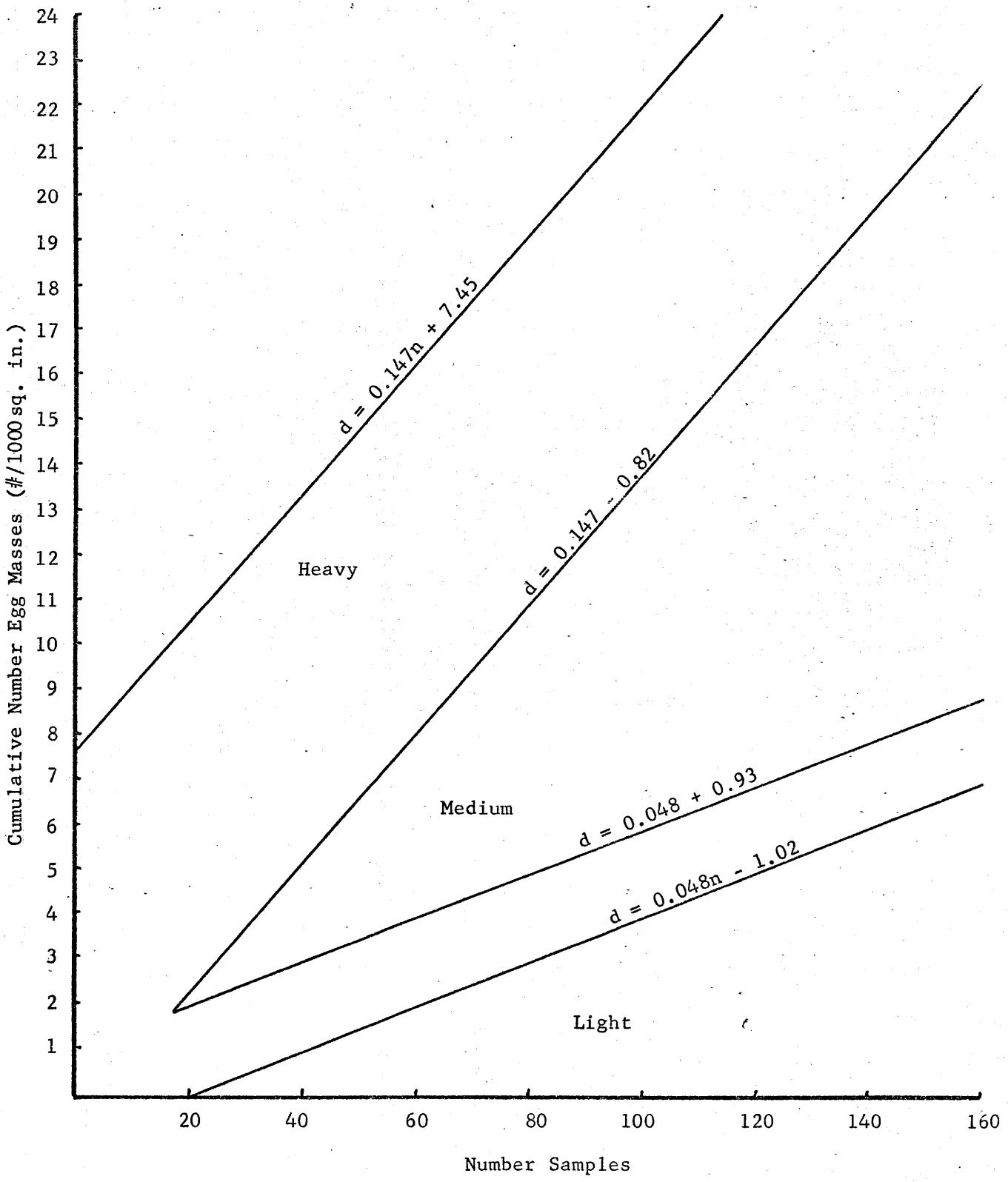

Figure 23.--Sequential graphs for sampling egg populations of the tussock moth.

Table 7.--Sequential sampling plan for Douglas-fir tussock moth egg masses (based on 5 samples per tree in a 1:3:1 combination)

Trees	Egg masses per 1,000 square inches					
	Light	vs	Medium	Medium	vs	Heavy
1	-	Continue sampling	-	-	Continue sampling	9
2	-		-	-		9
3	-		-	-		10
4	-		2	2		11
5	0		3	2		12
6	0		3	3		12
7	0		3	4		13
8	0		3	5		14
9	1		4	5		15
10	1		4	6		15
11	1		4	7		16
12	1		4	8		17
13	2		5	8		17
14	2		5	9		18
15	2		5	10		19
16	2		5	10		20
17	3		6	11		20
18	3		6	12		21
19	3		6	13		22
20	3		6	13		22
21	5		7	14		23
22	5		7	15		24
23	5		7	15		25
24	5		7	16		25
25	6		8	17		26

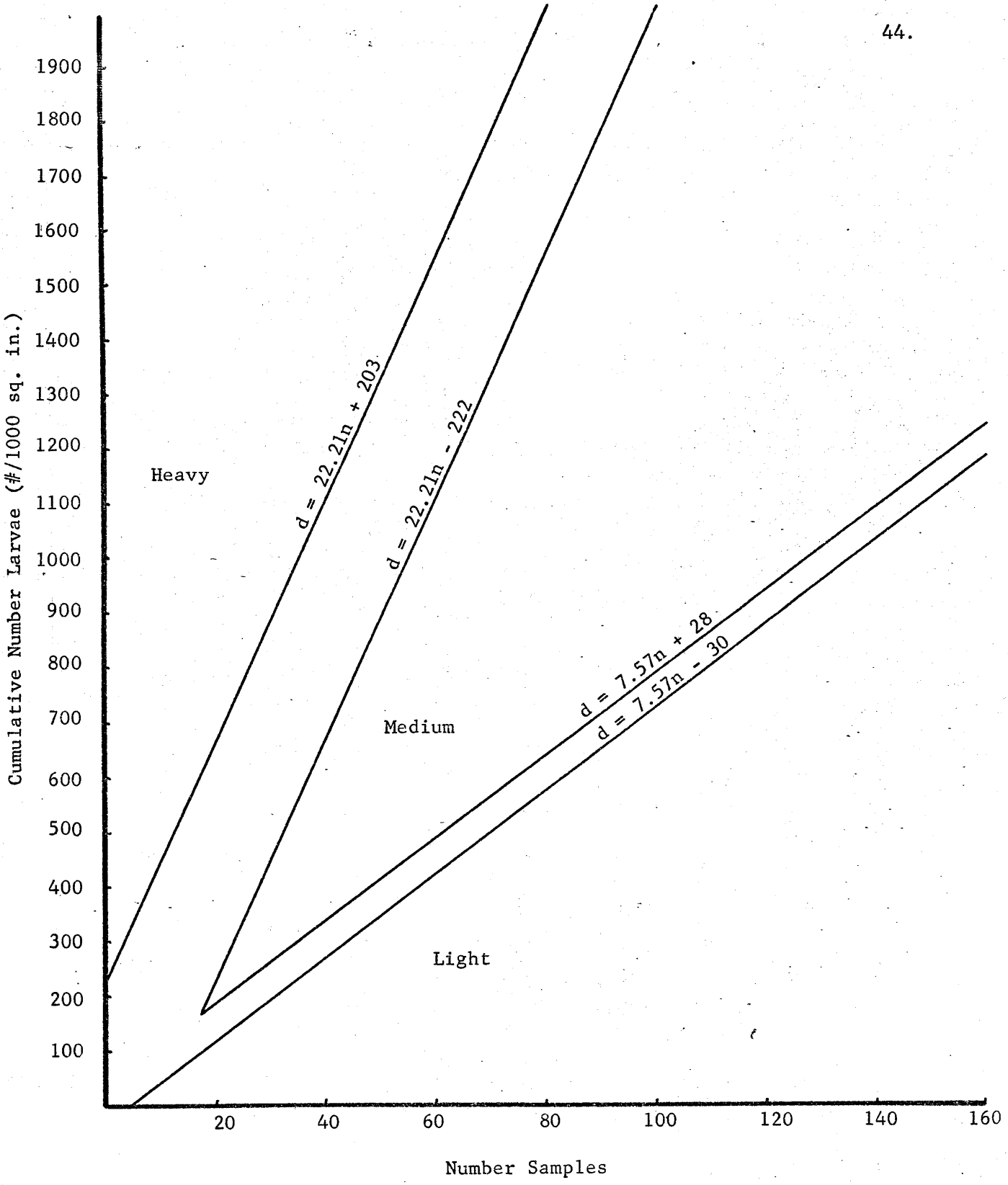

Figure 24.--Sequential graphs for sampling larval populations of the tussock moth.

Table 8.--Sequential sampling plan for Douglas-fir tussock moth larvae. (Based on 3 mid-crown samples per tree)

Trees	Larvae per 1,000 square inches					
	Light	vs	Medium	Medium	vs	Heavy
1	-	Continue sampling	-	-	Continue sampling	270
2	15		-	-		336
3	38		-	-		403
4	61		-	-		470
5	84		-	-		536
6	106		164	178		603
7	129		187	244		669
8	152		210	311		736
9	174		232	378		803
10	197		255	444		869
11	220		278	511		936
12	242		301	578		1003
13	265		323	644		1069
14	288		346	711		1136
15	311		369	777		1202
16	333		391	844		1269
17	356		414	911		1336
18	379		437	977		1402
19	401		459	1044		1469
20	424		482	1111		1536
21	447		505	1177		1602
22	470		528	1244		1669
23	492		550	1310		1735
24	515		573	1377		1802
25	538		595	1444		1869

Population Analyses

Most information collected over the course of the sampling studies proved to be reliable population estimates well within the 20 percent level of accuracy. Egg data, which had a standard error over 30 percent of the mean and which were obviously not an adequate sample of that stage, were the only exception. Because of the precision with which larval populations were estimated on most blocks, the data are quite amenable to further analysis of population change.

Partial life tables

Life tables are an effective numerical method of recording decline in an insect population and the mortality factors responsible. In this study continuous sampling of the tussock moth through the summer provided an accurate fix on the population at several different times in the generation. After the egg stage, for which the sample was unreliable, the number of larvae declined significantly from one measurement period to the next. It was then possible to estimate causes of mortality and their approximate impact from data supplied by those studying virus epizootiology in the same population. By compiling actual field population data and mortality estimates into the same table, a particularly clear accounting of trends and mortality impacts can be shown (Table 9). The 1x column contains number of living insects per 1,000 square inches of branch area after weighting samples according to the distribution of branch surface in white fir. Another figure was substituted for the unreliable egg measurement based on mortality estimates that occurred before the first larval sample.

Virus disease accounted for 41 percent of the decline recorded in the life table, which was the highest mortality from any single factor. Miscellaneous losses presumably can be attributed mostly to natural dispersal and predation. The loss of new foliage by freezing destroyed the favored food of early instar larvae so that starvation was probably a factor for some larvae. Dispersal by ballooning or wandering is very common among the early instars particularly when suitable food is not available so it would be difficult to separate death by starvation from hazards involved with dispersing even though dispersal may have been encouraged by poor food quality.

Two ichneumonid parasites were responsible for most of the larval parasitism recorded. These are yet unidentified. Their impact on the population may have been considerably greater than indicated by the life table since female adults of this family are known to feed on other larvae before ovipositing. This would be a predatory type of action by the adult parasite that could not be determined from larval rearings. Several small hymenopterous egg parasites were common in most egg masses, but none of them has yet been determined.

Life table data are illustrated graphically in Figure 25 as survivorship curves and corresponding death rates. For most of the larval stage total mortality occurred at a rather constant rate. Moreover, there seemed to be little variation through the season among the component mortality rates of disease, parasitism, and miscellaneous.

Table 9.--Partial life table for 1966 generation of Douglas-fir tussock moth at Corral Creek, California

Sample date	x Age interval	l_x No. alive at start of x (#/1,000 sq.in.)	d_xF Factors responsible for d_x	d_x No. dying during x	$100q_x$ d_x as percentage of l_x
May 24 - June 3	Eggs	47.5 (62.0)*	Virus disease	6.2	10
			Egg parasites	7.4	12
			Larval parasites	1.2	2
			Subtotal	14.8	24
June 12-17	1st larval sample (1st instar)	47.1	Virus disease	13.2	28
			Parasites	4.2	9
			Miscellaneous	18.0	38
			Subtotal	35.4	75
June 27-30	2nd larval sample (1st, 2nd, and 3rd instars)	11.7	Virus disease	4.6	39
			Parasites	1.0	9
			Miscellaneous	2.2	19
			Subtotal	7.8	67
July 11-15	3rd larval sample (3rd, 4th, and 5th instars)	3.9	Virus disease	1.4	35
			Parasites	0.3	9
			Miscellaneous	0.9	23
			Subtotal	2.6	67
July 25-27	4th larval sample (5th and 6th instars)	1.3	Virus disease	0.2	19
			Parasites	0.1	7
			Summary of Mortality:		
			Virus disease	25.6	41.3
			Egg parasites	7.4	11.9
			Larval parasites	6.8	11.0
			Miscellaneous	21.1	34.0
			TOTAL	60.9	98.2

*Corrected value calculated by adding estimated early mortality to first larval sample

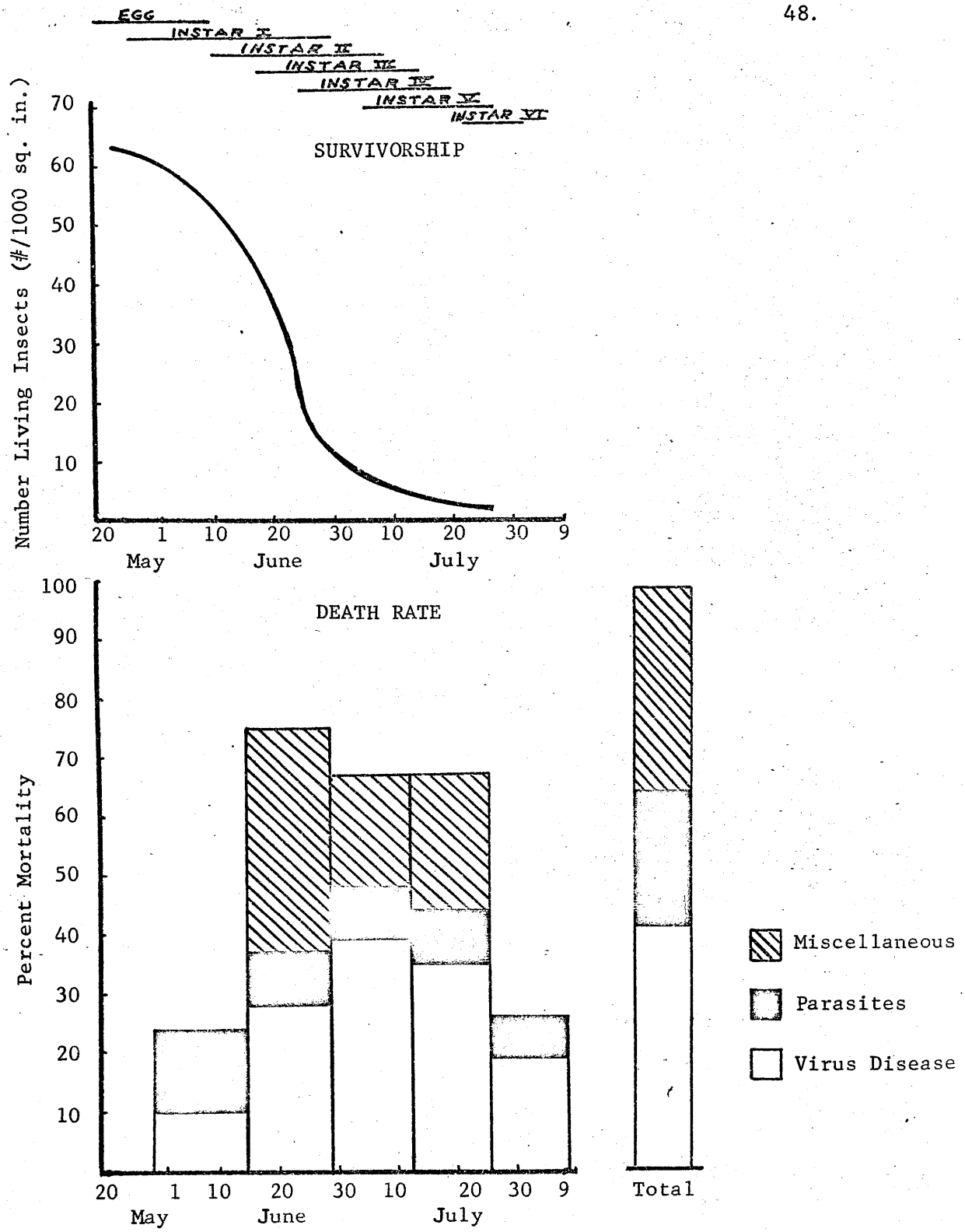

Figure 25.--Survival and death rate of tussock moth larvae at Corral Creek in 1966.

Analysis of survival

Study blocks established in the infestation were useful both for obtaining numerous estimates of variance in developing sampling techniques and for serving as replicates that could be treated and analyzed as separate populations. Each block was assumed to be an entity with its own population and characteristics. Based on information now available, this seems to be a fairly realistic assumption in spite of a certain amount of immigration and emmigration of larvae in the block. Survival and mortality data from eight different blocks, now treated as eight populations, permit regression analysis of certain processes that, otherwise, could only be accomplished with data from eight different generations. Results from analyzing several different populations in one year, however, do not include the effects of factors that are variable from year to year. Consequently this method should not be regarded as a substitute for the analysis of different generations, but only a technique for fully exploiting the data from one year.

A generally poor egg sample and the eventual outbreak collapse before pupation prevented any analysis of the entire generation. Yet, data from the four larval measurements can be examined to see if any useful patterns exist.

Age interval survival, or S-values, of larval populations from one sample period to the next were investigated using the expression

$$S = S_S \times S_M \times S_L \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

where S is proportion of larvae surviving after four measurements and S_S, S_M and S_L are respective survival estimates of small, medium, and large larvae. Survival in each block was respectively estimated for these values from weighted sample means ($N_{1L \cdots 4L}$) by: $S = N_{4L}/N_{1L}$; $S_S = N_{2L}/N_{1L}$; $S_M = N_{3L}/N_{2L}$ and $S_L = N_{4L}/N_{3L}$ (Table 10). The objective was to determine which period of larval development most strongly influenced the ultimate survival estimate. This is the technique described by Watt (1963) and Morris (1963), but which has been modified here somewhat because of incomplete generation data.

Table 10.--Age interval survival of larvae on study blocks

Block	S_S	S_M	S_L	Total
1	.562	.276	.320	.050
2	.043	.560	.143	.003
3	.077	.169	.200	.026
4	.588	.210	.600	.074
5	.520	.583	.357	.108
6	.187	.256	.516	.025
7	.261	.556	.250	.036
8	.136	.391	.111	.006
Total	.248	.333	.333	.028

A series of simple linear regressions were calculated to determine which of the age interval survivals (S_S, S_M, S_L) in the expression contributed most to variation in total survival (S). These results are shown in Table 11. Survival of both small and large larvae was significantly related to total survival. Of these, survival of small larvae seemed to have the most important influence on ultimate survival. As Watt (1964) has explained, correlations of this type contain a source of bias because the variables are not independent, nevertheless they are useful as a tool in studying populations to determine which variables are most influential.

Regression of population density on survival failed to show any significant relationships, but some trends seemed apparent. For example, three of the regressions had a negative correlation and a slope, "b", of less than one which implies an inverse relationship between population size and survival. Eight points are a rather small number of values with which to show significant relationships; more than likely, true density dependence could be demonstrated by examining a larger number of blocks than was possible in this study.

Table 11.--Regression statistics of larval survival (S) and population density (N)

Dependent variable	Independent variable	r^2	r	b
S	S_S	.740	.860**	1.079
S	S_M	.056	-.236	-0.597
S	S_L	.615	.784**	1.617
S	N_{1L}	.201	-.448	- .867
S_S	N_{1L}	.259	-.509	- .784
S_M	N_{2L}	.143	-.378	- .216
S_L	N_{3L}	.155	.394	.290

** Significant at the .01 level.

Analysis of mortality

An alternative method of analysis has been developed by Varley and Gradwell (1960) which uses mortality estimates instead of survival. Their technique is especially useful for identifying the most effective mortality factors or "key factors" that act on the population. Unfortunately, information on the impact of mortality factors in each block is not available so this method cannot be applied in its usual sense. However, it can be used, in a way that is statistically similar to the analysis of survival data, to identify the larval stage at which a key factor is operating. The basic unit for analysis is the k-value which is the difference between the logarithm of population estimates before and after mortality has taken place. In other words, the k-value is the logarithm of the quotient resulting from dividing population density before mortality by population density after mortality. Applying Varley and Gradwell's method to the tussock moth data

$$K = k_S + k_M + k_L \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

where K is total larval mortality and k_S, k_M and k_L are interval mortalities of small, medium and large larvae occurring between each measurement period.

Employing the same approach that was used in the analysis of survival, regressions were calculated to determine which mortalities (k_S, k_M and k_L) contributed most to variation in total mortality (K). As expected, the results shown in Table 12 are similar to those calculated for survival since the same original data were used to calculate both K-values and S-values. The regression of small larval mortality (Table 12) on total mortality (K) was even better than the comparable analysis of survival (Table 11). There also seemed to be a density dependent action between small larvae and total mortality, but this could not be significantly demonstrated with data from only eight blocks.

Table 12.--Regression statistics of larval mortality (K) and population density (N)

Dependent variable	Independent variable	r^2	r	b
K	k_S	.889	.943***	1.397
K	k_M	.021	.144	0.427
K	k_L	.602	.766**	1.862
K	N_{1L}	.366	.605*	1.378
k_S	N_{1L}	.258	.508	0.781
k_M	N_{2L}	.143	.379	0.664
k_L	N_{3L}	.154	-.393	-0.533

*** Significant at the .001 level
** Significant at the .01 level
* Significant at the .10 level

Results of these analyses suggest that disappearance of larvae in the first sampling stages had the strongest influence on ultimate population density in each block. Life table data show that disease and parasitism together took an estimated 37 percent of the population during this period, but another 38 percent mortality was unaccounted for (Table 9). Presumably, loss from dispersal, predation, and starvation made up the remainder. Since first instar larvae are especially mobile, a logical explanation is that dispersal was responsible for a large loss and that this was directly related to population density in each block as implied by some of the analyses. However, virus incidence may also have been density dependent so that more larvae proportionately were being killed by disease on blocks with a

high population than on blocks with a low population. Other general evidence from virus epizootiological studies in the outbreak indicates, in fact, just such a pattern may have occurred. These analyses emphasize the need for an accurate estimate of mortality factors and their impact on the population in each unit and not just the area as a whole. Systematic measurements of this type are extremely important and should be given high priority in future population studies, especially when the impact of disease is under investigation or being field tested.

DISCUSSION AND RECOMMENDATIONS

Evidence from this study shows that reasonably precise estimations can be made of outbreak populations of the tussock moth. Calculations also indicate that the precision of estimate obtained here is probably adequate for life table work and other population analyses. Eggs have been sampled in other investigations, but there are no published accounts of precise sampling of tussock moth larval stages.

Findings reveal that larvae may provide a more accurate estimate of population size and require less sampling than eggs. Especially in the case of low populations, larvae, which are more uniformly distributed through the crown, should be encountered much more frequently on a sample than egg masses. Furthermore, larvae can be sampled satisfactorily from the mid-crown of the tree only and, thus, enable larger trees to be examined with the same aerial equipment than would be possible if the tops of trees also had to be sampled. In the event only one fix is made on the population in a season, timing of the larval sample becomes critical because numbers of larvae decline sharply after eclosion. Careful notes should be kept of the age of larvae which are sampled so that in subsequent analyses populations are compared at the same stage of development.

In the course of reviewing this manuscript, individuals have made several valuable suggestions which, after further study, could modify the sampling procedures presented. For example, adjustment of all samples to 1,000 sq.in. was done only to correct samples of a different size to a common unit. Relating insect numbers to a measurable unit may also be necessary to convert sample means to absolute population values expressed on an acre basis. However, adjusting samples to a common unit can be a tedious task in the field, especially when insect counts must be accumulated in a sequential sampling plan. There is some evidence that, in the limited size range of the samples examined, tussock moth numbers are not at all related to the size of the sample. If such is the case, adjustment of each sample to 1,000 square inches of branch area would not be necessary and counts could be interpreted in terms of insects per branch sample. This possibility should definitely be explored further.

The results presented in this report are preliminary and caution should be taken in interpreting them too freely. The sections on absolute populations and population analyses especially should be regarded with restraint until substantial data can be collected that establish the proper relationships. Nevertheless, these sections illustrate techniques that should be considered in planning and carrying out further studies of the tussock moth once the sampling problems are solved. Additional studies on sampling are still required to perfect the procedures already outlined. Some of these are as follows:

1. Develop methods for sampling endemic populations.

2. Explore the use of standard branch sample as the common unit for relating insect counts.

3. Perfect a sequential sampling plan for practical field use.

4. Experiment with transformations suitable for normalizing sample data.

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